

Minicatalog™ 2007

**The Protein
Experts**

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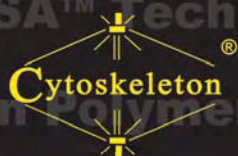
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New Products for 2007

Rac G-LISA™ Activation Assays

Cytoskeleton Inc. introduced the revolutionary Rho G-LISA™ Activation Assay last year to a great reception from research scientists. This year we are expanding the product line to include Rac1,2,3 and Rac1 specific G-LISA™ Activation Assays.

The new Rac G-LISA™ Activation Assays can be performed in under 3 hours compared to the usual 1.5 days for the traditional pull-down version (e.g. Cat.# BK035). G-LISA™ assays are more reliable than pull-down assays and since you only need a few percent of the material required for pull-downs, the G-LISA™ format will allow you to do more assays (e.g.

duplicates) than you could before, allowing you to test more conditions and get conclusive results quicker. For more detailed information on the other G-LISA™ assays, see pages 10 & 17.

Rac G-LISA™ uses

- Rac signaling pathway studies
- Rac activation assays with primary cells
- Studies of Rac activators and inactivators
- Rac activation assays with limited material
- High throughput screens for Rac activation and inactivation

Characteristics of the Rac G-LISA™ assays

- Rapid (<3 h)
- Simple
- Reliable
- Quantitative
- HTS compatible
- Requires only 1-5% of the material needed for a pull-down assay

The G-LISA™ Advantage

The G-LISA™ assay (patent pending) is based on the same principle as the traditional pull-down assay but we have moved the assay into a 96-well plate (see Fig. 1 & Fig. 2D). Because of this, G-LISA™ assay results are comparable to a conventional pull-down assay. You can easily switch from the pull-down approach to G-LISA™ and continue getting similar results, with the difference being that your experiments will be faster, simpler and you will get more consistent results. See page 10 for more information.

Table 1. G-LISA™ vs Pull-down assays

	G-LISA™	Pull-down Assay
Works on a wide variety of species and cell types	✓	✓
Can handle large numbers of samples	✓	✗
Works on small amounts of cell material	✓	✗
Fast	✓	✗
Quantitative	✓	✗
Reproducible	✓	✗

The G-LISA Principle

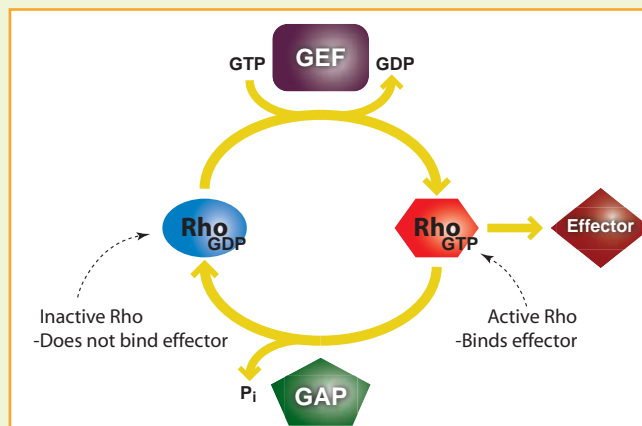


Figure 1. The Rho family GTPase cycle. Rho, like other small G-proteins cycles between an inactive GDP-bound state and an active GTP-bound state. Effectors only bind to the active conformation. In both conventional pull-down assays and in G-LISA, an effector domain is used to separate the active G-protein from the inactive and the amount of active G-protein is measured. In the G-LISA™ assay, the effector domain is covalently coupled to 96-well plates and the entire assay is performed in the wells.

Customer Feedback

In the first year since its introduction, the G-LISA™ assay was successfully used by many scientists. The assay was easily performed by labs with either knowledge of the traditional pull-down format or no prior Rho assay experience. Publications containing the technique are:

1. Higashibata A, Imamizo-Sato M, Seki M, Yamazaki T, and Ishioka N. 2006. Influence of simulated microgravity on the activation of small GTPase Rho involved in cytoskeletal formation – molecular cloning and sequencing of bovine leukemia-associated guanine nucleotide exchange factor. BMC Biochemistry, 7, (19), p1-9.

2. Woods A. and Beier F. 2006. RhoA/ROCK signaling regulates chondrogenesis in a context-dependent manner. J. Biol. Chem. 281, (19), p.13134-13140.

3. Warren JC, Rutkowski A. and L. Cassimeris. 2006. Infection with replication-deficient adenovirus induces changes in the dynamic instability of host cell microtubules. Mol. Biol. Cell, 17, p3557-3568.

Success with a wide variety of cell types including:

- Primary Cells
- Tumor Cells
- Endothelial Cells
- Epithelial Cells
- Fibroblasts
- Macrophages

See the website for a complete list of cell types used with G-LISA™: www.cytoskeleton.com/g-lisatechtip.htm

Product	Amount	Cat. #
Rac1 G-LISA™ Activation Assay, luminescence.	96 assays	BK126
Rac1,2,3 G-LISA™ Activation Assay, colorimetric.	96 assays	BK125
RhoA,B,C G-LISA™ Activation Assay, colorimetric.	96 assays	BK123
RhoA G-LISA™ Activation Assay, luminescence or colorimetric	96 assays	BK121 or BK124

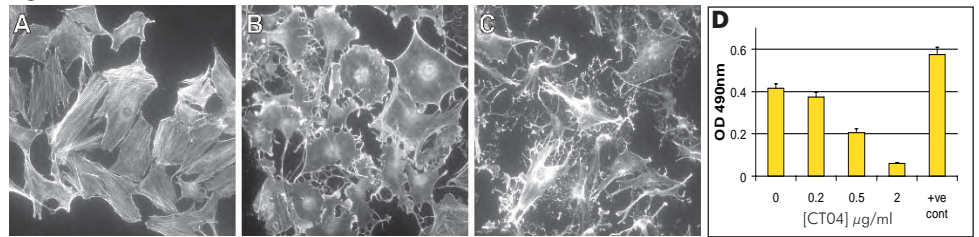
New Products for 2007

Cell Permeable Rho Inhibitor

The exoenzyme C3 Transferase from *Clostridium botulinum* is commonly used to selectively inactivate the GTPases RhoA, RhoB, and RhoC, both *in vivo* and *in vitro* by ADP-ribosylation on Asn 41. Cytoskeleton, Inc. modified this protein by covalently linking a proprietary cell penetrating peptide to the protein. The cell penetrating moiety allows rapid and efficient transport through the plasma membrane. Once in the cytosol the cell penetrating moiety is released, thereby activating C3 Transferase.

In contrast to Rho inactivation by siRNA which is slow (24h) and disrupts cell metabolism and shape, the Cell Permeable Rho Inhibitor, Cat.# CT04, is a rapid and highly potent inhibitor, being able to inactivate Rho within 2h at less than 2.0 $\mu\text{g/ml}$.

Figure 2



Cell permeable C3 transferase disrupts stress fibers in Swiss 3T3 cells and can be titrated to induce either moderate or robust phenotypes. A - Control, B - plus 1 $\mu\text{g/ml}$ for 2h in medium without serum (moderate phenotype), C - plus 2 $\mu\text{g/ml}$ for 4h in medium without serum (robust). Notice the lack of stress fibers in B, and retracted phenotype in C. D - Quantitative analysis of Rho inhibition by using the RhoA G-LISA Activation Assay (Cat. # BK124) on cell lysates after CT04 treatment for 4h with the noted concentrations of CT04, performed in serum free medium.

Product	Amount	Cat. #
Cell Permeable Rho Inhibitor	1 x 20 μg 5 x 20 μg	CT04-A CT04-B

Rac1 Specific Antibody

The anti-Rac1 antibody (Cat. # ARC03) is a mouse monoclonal antibody that is specific for Rac1 and does not recognize Rac2, Rac3, Cdc42 or other small GTPases. Rac1 is expressed in a large number of different cell types. Most commercially available Rac1 antibodies will cross react with Rac2 and/or Rac3 and Cdc42 (Figure 3a).

Western blot analysis: ARC03 does not cross-react with Rac2, 3 or Cdc42 (upper left blot), while all other commercially available Rac1 antibodies crossreact with GTPases other than Rac1. The blot was probed with a 1 $\mu\text{g/ml}$ (1:500 dilution) of ARC03. Other blots were probed according to manufacturers' protocols. The SDS-PAGE gels were loaded with 25 ng His-Rac1 (lane 1), His-Rac2 (lane 2), GST-Rac3 (lane 3), GST-Cdc42 (lane 4) and 50 μg of platelet extract (lane 5).

Immunofluorescence: Swiss 3T3 cells were fixed with methanol and probed with 10 $\mu\text{g/ml}$ ARC03 prior to probing with 1:500 of anti-mouse rhodamine secondary antibody.

Figure 3a, Western blot comparison

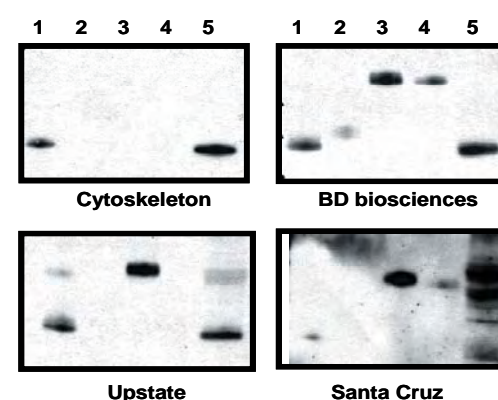


Figure 3b, Immunofluorescence



Swiss 3T3 cell stained with ARC03.

Product	Amount	Cat. #
Rac1 Specific Antibody	2 x 50 μg 6 x 50 μg	ARC03-A ARC03-B

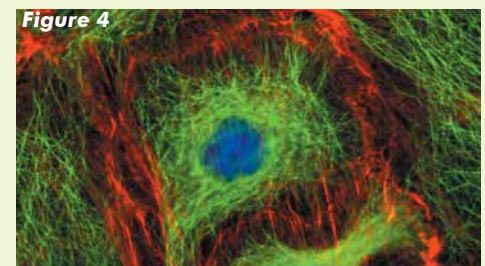
New Proteins

- **Pre-formed Actin Filaments** (see p.4 and 13)
- **Cardiac Myosin** (see p.5 and 12)
- **Rap1a and Rap1b Small G-proteins** (see p.18)
- **X-rhodamine Tubulin** (see p.21)
- **Rhodamine Fibronectin**

Use rhodamine fibronectin to follow a cell's path by the non-fluorescent track in leaves in a rhodamine fibronectin coated glass slide or in a 3D matrix. Visit www.cytoskeleton.com/fnr01.html for more information.

Cytoskelfix™ Cell Fixative

A new cell fixative that is ideal for fixing actin and tubulin based structures in fine detail. Traditionally microtubule structures have been fixed with cold methanol, whereas F-actin structures have been fixed with paraformaldehyde or a similar cross linker. Cytoskelfix™ reagent allows you fix both at the same time which reduces time and costs, and assists in visualizing both types of structures in the same cell without losing clarity. Recent publications have shown this reagent to enhance the visualization of F-actin and microtubule associated proteins. Citations: Robinson RW and Snyder JA. 2004. An innovative fixative for cytoskeletal components... *Histochem. Cell Biol.* **122**, 1-5. Robinson RW and Snyder JA. 2005. *Protoplasma*, **225**, 113-122.



Actin stained with rhodamine phalloidin (red), tubulin stained with anti-tubulin (Cat.# ATN02) plus a fluorescein secondary antibody (green). Cytoskelfix is a trademark R&D Enterprises Inc.

Product	Amount	Cat. #
Cytoskelfix™ Cell Fixative	1 x 100 ml 3 x 100 ml	CSK01-A CSK01-B

Actin Proteins

About Actin Products

The eukaryotic actin cytoskeleton is integrally involved in many physiological and cellular processes including muscle contraction, cell shape, cell motility, cell division and signal transduction. In diseased cells actin is required for the intracellular propagation of many bacterial and viral pathogens and is involved in metastatic invasion.

In order to carry out its cellular functions the actin cytoskeleton is required to be a highly dynamic structure, capable of rapid response to a changing cellular environment. Actin dynamics are stringently regulated by over 200 actin binding proteins,

divisible into at least 50 distinct classes. Such a large number of regulatory proteins serves to underline the importance of intracellular actin regulation and to highlight the necessity of understanding these regulatory pathways.

Higher eukaryotes express six different actin isotypes that are tissue specific and have molecular weights of approximately 42 kDa. These are striated muscle actins (skeletal and cardiac), smooth muscle actins (vascular and visceral), and non-muscle actins (β and γ isotypes). All isotypes are virtually identical between mammalian species and are

highly homologous between isotypes. For this reason, rabbit skeletal muscle actin has become a "universal substrate" in actin research. There is, however, mounting evidence that actin isotypes show differing specificities to actin binding proteins.

Cytoskeleton, Inc. offers a large selection of highly pure and biologically active actin proteins and actin regulatory proteins (see NEW Products below). Cytoskeleton also offers a range of Actin Biochem Kits™ that allow researchers easy access to some of the most powerful *in vivo* and *in vitro* assays employed in the field.

Purified Actins

To help researchers answer the specific actin-related questions they may have, Cytoskeleton provides actins from several sources. These include:

- Non-muscle actin
- Cardiac muscle actin
- Skeletal muscle actin and pre-formed F-actin
- Smooth muscle actin

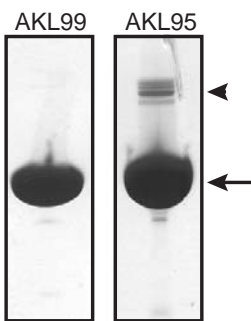
Purity:

The actin proteins are available in either >99% or >95% purity, where the >95% purity preparations offer an economical alternative to the highest purity preparations.

Uses for purified actins:

- Measure gelsolin severing activity (ref. e.g. Arora *et al.* J. Biol. Chem. 271:20516-20523, 1996)
- Measure Arp2/3 or formin nucleation activity (ref. e.g. Humphries *et al.* J. Cell Biol. 159:993-1004, 2002)
- Small molecule drug discovery (ref. e.g. Bai *et al.* J. Biol. Chem. 277:32165-32171, 2002)
- Binding assay for F-actin binding proteins (ref. e.g. Chen *et al.* Mol. Biol. Cell 10:4327-4339, 1999)
- Binding assay for monomer binding proteins (ref. e.g. Eckley *et al.* Mol. Biol. Cell 14:2645-2654, 2003)
- F-actin bundling assay (ref. e.g. Loomis *et al.* J. Cell Biol. 163:1045-1055, 2003)
- F-actin depolymerization (ref. e.g. Vartiainen *et al.* Mol. Biol. Cell 13:183-194, 2002)

Figure 1. Purities of rabbit skeletal muscle actin protein. 100 μ g of >99% pure (AKL99) and >95% pure (AKL95) rabbit skeletal muscle actin were run on SDS-PAGE gels and stained with coomassie blue. In polymerization tests AKL99 produces



>90% F-actin and AKL95 produces >80% F-actin. The arrow indicates actin protein, the arrowhead an α -actinin contaminant (115 kDa). The minor impurities in the purified actins are predominantly actin binding proteins such as α -actinin and gelsolin.

Labeled Actins

Cytoskeleton provides highly pure and biologically active biotinylated, rhodamine and pyrene actins. These labeled actins have many uses such as:

- Following actin dynamics in living cells (Rhodamine actin)
- Visualization of microfilaments in myosin motility assays (Rhodamine actin)
- Actin binding assays using SPA beads or streptavidin coated plates (Biotinylated actin)
- *In vitro* actin polymerization assays (Pyrene actin)

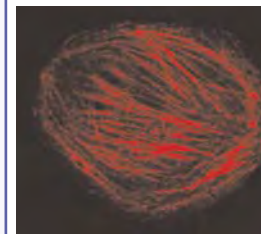


Figure 2.

Rhodamine actin has been microinjected into CHO cells. The labeled actin rapidly incorporates into the cellular actin cytoskeleton and allows real time observation of actin dynamics.

Actin Proteins & Labeled Actins

Actin Protein	Cat. #	Amount
Actin Protein Rabbit skeletal muscle, >99% pure	AKL99-A	4 x 250 μ g
	AKL99-B	2 x 1 mg
	AKL99-C	5 x 1 mg
	AKL99-D	10 x 1 mg
	AKL99-E	20 x 1 mg
	AKL99-XL	Large quantities
Actin Protein Rabbit skeletal muscle, >95% pure	AKL95-B	1 x 1 mg
	AKL95-C	5 x 1 mg
	AKL95-D	10 x 1 mg
	AKL95-E	20 x 1 mg
	AKL95-XL	Large quantities
Actin Protein Bovine cardiac muscle, >99% pure	AD99-A	1 x 1 mg
	AD99-B	5 x 1 mg
	AD99-C	20 x 1 mg
Actin Protein smooth muscle, chicken gizzard >99% pure	AS99-A	1 x 1 mg
	AS99-B	5 x 1 mg
	AS99-C	20 x 1 mg
Actin Protein Human platelet, non-muscle, >99% pure	APHL99-A	2 x 250 μ g
	APHL99-C	1 x 1 mg
	APHL99-E	5 x 1 mg
Actin Protein Human platelet, non-muscle, >95% pure	APHL95-C	1 x 1 mg
	APHL95-D	5 x 1 mg
	APHL95-E	10 x 1 mg
Biotinylated Actin Protein Rabbit skeletal muscle	AB07-A	5 x 20 μ g
	AB07-C	20 x 20 μ g
NEW Pre-formed Actin Filaments , Rabbit skeletal muscle (for other actins in this format please inquire).	AKF99-A	1 x 1 mg
	AKF99-B	5 x 1 mg
Pyrene Actin Protein Rabbit skeletal muscle	AP05-A	1 x 1 mg
	AP05-B	5 x 1 mg
Rhodamine Actin Protein Human platelet, non-muscle	APHR-A	4 x 10 μ g
	APHR-C	20 x 10 μ g
Rhodamine Actin Protein Rabbit skeletal muscle	AR05-B	10 x 20 μ g
	AR05-C	20 x 20 μ g

Actin Binding Proteins

The actin cytoskeleton plus actin binding proteins together constitute over 25% of the protein in non-muscle cells. Hundreds of actin binding proteins (ABPs) have been identified. They can generally be grouped into the following types of ABPs:

Monomer Binding Proteins: Bind to and sequester G-actin, e.g. profilin (Cat. # PR01).

Capping Proteins: Cap one end of actin filaments, usually the barbed, fast growing end, to regulate dynamics (e.g. ezrin and CapZ).

Severing Proteins: Sever actin filaments, also often have capping function, e.g. gelsolin (Cat. # HPG5) and cofilin (Cat. # CF01).

Side Binding/Bundling Proteins: Bind to F-actin and may cause crosslinking, e.g. α -actinin

Actin Binding Proteins

(Cat. # AT01).

Motors and Miscellaneous ABPs: By far the largest group, includes myosins (Cat. # MY02, MY03 & MH01) and Arp 2/3 complex (Cat. # RP01).

Cytoskeleton's purified actin binding proteins are all in a ready to use format, highly pure (>80 to >95% purity) and have been carefully tested for biological activity.

Selected Product Citations:

AT01: Maul *et al.* 2003, *J. Cell Biol.* 160, 399-407

CF01: Idrissi *et al.* 2002, *Mol. Biol. Cell* 13, 4074-4087

HPG5: Wang *et al.* 2003, *J. Cell Biol.* 160, 565-575

MH01: Li *et al.* 2004, *J. Cell Sci.* 117, 3593-3604

MY02: Gallo *et al.* 2002, *J. Cell Biol.* 158, 1219-1228

PR01: Moseley *et al.* 2004, *Mol. Biol. Cell* 15, 896-907

RP01: Leng *et al.* 2005, *PNAS.* 102, 1098-1103

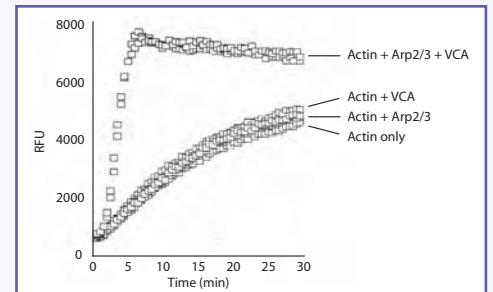


Figure 3. Actin polymerization stimulated by Arp2/3 and the WASP VCA domain. Kit BK003 was used to study the effects of Arp2/3 (Cat. # RP01) and the WASP VCA (Cat. # VCG03) domain on actin polymerization *in vitro*.

Actin Binding Proteins	Cat. #	Amount
α-Actinin Protein Rabbit skeletal muscle	AT01-A AT01-C	2 x 50 μ g 10 x 50 μ g
Arp2/3 Protein Complex Bovine brain	RP01-A RP01-B	2 x 50 μ g 6 x 50 μ g
Cofilin Protein Human recombinant	CF01-A CF01-C	1 x 100 μ g 4 x 100 μ g
Gelsolin Protein Human plasma	HPG5-A HPG5-B HPG5-C	4 x 25 μ g 8 x 25 μ g 20 x 25 μ g
NEW Myosin Cardiac Protein Bovine cardiac muscle	MY03-A MY03-B	1 x 1 mg 5 x 1 mg
Myosin: Heavy Meromyosin Chymotrypsin digest of rabbit muscle myosin II	MH01-A MH01-B	4 x 50 μ g 20 x 50 μ g
Myosin II Protein Rabbit skeletal muscle	MY02-A MY02-B	5 x 1 mg 20 x 1 mg
Profilin Protein Human platelet	PR01-A PR01-C	1 x 50 μ g 4 x 50 μ g
WASP VCA Domain GST Fusion Protein Human recombinant, binds and activates Arp2/3	VCG03-A VCG03-B	1 x 500 μ g 5 x 500 μ g

Actin & Actin Binding Protein Antibodies	Cat. #	Amount
Actin Antibody Affinity purified rabbit polyclonal, includes positive control	AAN01-A AAN01-B	1 x 100 μ g 3 x 100 μ g
Arp3 Antibody Affinity purified sheep polyclonal, includes positive control	AAR01-A AAR01-B	1 x 50 μ g 3 x 50 μ g
Cofilin Antibody Affinity purified rabbit polyclonal, includes positive control	ACFL02-A ACFL02-B	1 x 50 μ g 3 x 50 μ g
Fibrillarlin Antibody Mouse monoclonal	AFB01-A AFB01-B	1 x 100 μ g 3 x 100 μ g
Profilin Antibody Affinity purified rabbit polyclonal, includes positive control	APUF01-A APUF01-B	1 x 50 μ g 3 x 50 μ g
Sheep IgG Antibody, horseradish peroxidase labeled Affinity purified donkey polyclonal	SG01	1 x 0.5 mg
Sheep IgG Antibody, rhodamine labeled Affinity purified donkey polyclonal	SG02	1 x 0.5 mg

Actin Product Line Buffers & Reagents	Cat. #	Amount
Actin Polymerization Buffer (10 x stock) For the polymerization of G-actin to F-actin	BSA02-001	1 x 2 ml
ATP (100 mM stock solution) ATP is required for actin stability and polymerization	BSA04-001	1 x 1 ml
General Actin Buffer For resuspending & diluting G-actin protein	BSA01-001 BSA01-010	1 x 10 ml 1 x 100 ml
Phalloidin (rhodamine labeled) 14 μM in methanol Stabilizes and binds selectively to F-actin	PHDR1	1 x 500 μ l

Actin & Actin Binding Protein Antibodies

Cytoskeleton Inc is dedicated to providing high quality antibodies to actin and actin binding proteins. All our antibodies in this line of products are QC Max[™] tested. QC Max[™] antibodies have been tested for use in western blots, *in situ* and immunoprecipitation. This way, you know exactly what each antibody can be used for and you can make sure that you buy an antibody that is appropriate for you. If you are interested in the specifics about one of our QC Max[™] antibodies, go to our website or contact our customer service at tservice@cytoskeleton.com.

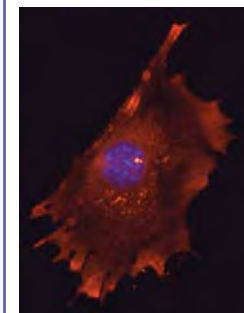


Figure 4. Immunofluorescent staining of a Swiss 3T3 cell using an anti-cofilin antibody (Cat. # ACFL02). The cofilin staining is shown in red. Blue color represents DAPI staining of the nucleus. Note the localization of cofilin in protrusions.

Actin Biochem Kits[™]

Cytoskeleton Inc offers a broad range of kits to monitor and test actin and actin binding protein function *in vivo* and *in vitro*. These include:

- Actin Binding Protein Spin-down Assays
- Actin Filament Biochem Kit[™]
- Actin Polymerization Biochem Kit[™]
- Cytoskeleton Isolation Biochem Kit[™]
- F-actin Visualization Biochem Kit[™]
- G-actin / F-actin *In Vivo* Assay Kit

See page 7 for more information on these kits.

Biochem Kits™

What Are Biochem Kits™?

Biochem Kit™ Lines

Biochem Kits™ are user friendly assays that can be divided into four main categories (see Table 1 below).

Table 1

Biochem Kit™ Category	Page
Actin Biochem Kits™	7
Tubulin Biochem Kits™	8
Phosphate Quantitation and Kinesin Motor Biochem Kits™	9
Signal Transduction Biochem Kits™, including G-LISA™ Biochem Kits™	10-11

The Biochem Kit™ Advantage

- Biochem Kits™ distill technically demanding assays into user friendly formats that provide reliable results for both experienced and novice researchers.
- Innovative approaches to assay development allow researchers access to greatly improved assay methodologies. For example, our NEW GAP assay kit eliminates the requirement for radioactive nucleotides, a problem with the more classical assay. Likewise, our NEW G-LISA™ kits introduces an ELISA type format to the classic G-protein activation assay. This innovation allows rapid throughput and superior quantitation of samples.
- Superior quality technical service from scientists with years of experience in the areas outlined in Table 1.
- Detailed protocols are provided, including an extensive trouble-shooting guide.

Uses for Biochem Kits™

Cytoskeleton's Biochem Kits™ can be used to assay a wide range of cytoskeletal and signal transduction events, both *in vivo* and *in vitro*. Examples of uses are:

- Monitoring actin dynamics *in vitro* and *in vivo* (see page 7)
- Monitoring microtubule dynamics *in vitro* and *in vivo* (see pages 8 & 14)
- Measuring the activities and regulation of small G-proteins (see pages 10-11 & 17)
- Monitoring the function of molecular motors (See pages 9 & 12-13)
- Testing the function and activities of ATPases, GTPases and other phosphatases (see page 9)

Biochem Kit™ Formats

An example of a typical Biochem Kit™ format is given below for the kinesin ATPase end-point assay (BK053).



Kit Contents

Each kit contains sufficient reagents for 1000 individual assays.

- CytoPhos™ reagent for P_i quantitation
- Kinesin motor protein control
- Pre-formed microtubules
- Kinesin reaction buffer
- ATP stock
- Paclitaxel
- Paclitaxel resuspension buffer
- Phosphate standard
- Detailed protocol with extensive trouble-shooting guide

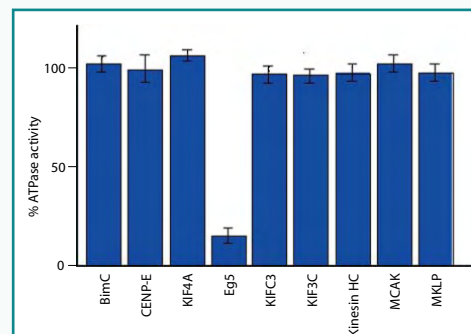


Figure 1. Kinesin ATPase end point assay. BK053 was used to screen a set of nine kinesin motors against the known kinesin inhibitor monastrol. The assay confirms the previously published results that monastrol is a specific inhibitor of Eg5 kinesin. The histogram data represents kinesin microtubule activated ATPase activity of kinesins in the presence of 100 μM monastrol. The assay CV value is 6%. IC₅₀ for monastrol inhibition of Eg5 was determined to be 10 μM using Biochem Kit™ BK060, in agreement with previously published data.

What's New In 2006

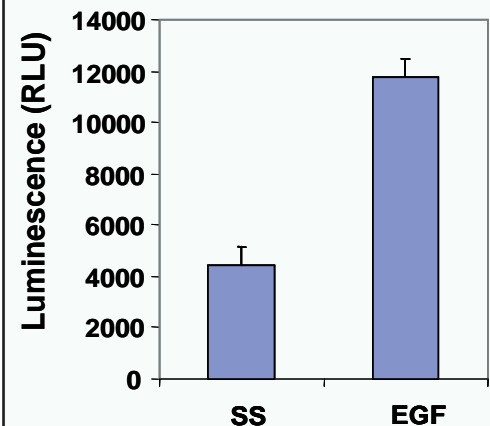
G-LISA™ kits (BK125-126): ELISA based Rac activation assays that are faster, more reliable and require less material than the conventional pulldown activation versions (see pages 2, 10 & 17).

Since their introduction G-LISA™ assays have made a significant impact on the research generated in this area. The new Rac1,2,3 (BK125) and Rac1 specific (BK126) versions are likewise very robust and accurate assays that generate data five times faster than conventional formats. Researchers have used the assays to measure activation in numerous cell types including,

- Primary Cells
- Tumor Cells
- Endothelial Cells
- Epithelial Cells
- Fibroblasts
- Macrophages

The broad application of cell types indicates a flexible assay that can be exploited in many experimental designs. For example, there is potential for these assays to be used in drug discovery to develop compounds that reduce inflammation or inhibit metastasis.

Results obtained with Swiss 3T3 cells treated with EGF to stimulate Rac Activation and measured with the Rac1 Activation Assay (BK126).



Results are quantitative in the range of 0.01 to 1.0ng of Rac-GTP bound protein which means that only 1 to 10μg of total protein as cell lysate is required. Such low amounts of required protein mean that even small quantities of primary cells can be assayed accurately. SS = Serum starved. [EGF] = 10ng/ml.

Drug Discovery Applications

Many Biochem Kits™ can be adapted to drug discovery applications in high throughput screen (HTS) format. The drug discovery section of this Mini-catalog outlines HTS assay formats (see pages 14-17).

For more information about setting up HTS assays, inquire to tservice@cytoskeleton.com.

Actin Biochem Kits™

Actin Binding Protein Spin-Down Assay™ (BK001 & BK013)

This simple co-sedimentation assay allows the identification and characterization of proteins that bind to actin filaments and hence exhibit APB characteristics. The kit is available in muscle and non-muscle actin formats.

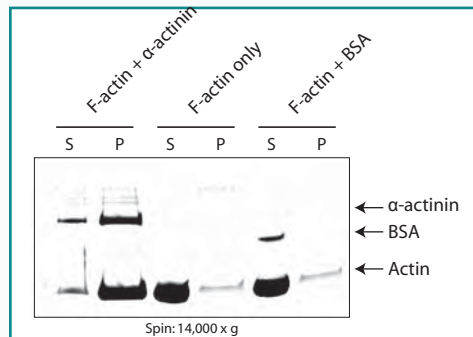


Figure 1. Actin bundling assay using kit BK001. F-actin was incubated alone or together with α -actinin or BSA. Bundled F-actin was pelleted by a 14,000 x g centrifugation and pellets (P) and supernatants (S) were run on an SDS-PAGE gel. Only in the presence of the F-actin bundling protein α -actinin is actin pelleted at this centrifugation speed.

Selected product citations for BK001 and BK013:
BK001 Zhai *et al.* 2001, *J. Biol. Chem.*, 276, p36163-36167.
BK013 Gohla *et al.* 2005, *Nat. Cell Biol.*, 7, 21-29.

Cytoskeleton Isolation Kit (BK039)

Used to simultaneously isolate all three cytoskeletal filament networks (microfilaments, microtubules and intermediate filaments) and their associated proteins. Useful in the characterization of proteins associated with the cytoskeleton. The kit includes drugs that let you determine which cytoskeletal network your protein of interest associates with.

Actin Polymerization Biochem Kit™ (BK003)

Employs fluorescent pyrene actin to monitor actin polymerization. It is very useful in the characterization of ABPs as the polymerization curve shape is often indicative of a given type of ABP. For example, a nucleating protein will give a characteristically steep polymerization curve while a monomer binding protein will give a shallow curve.

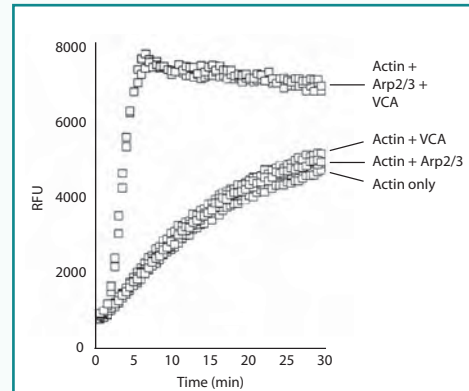


Figure 2. Use of BK003 to monitor actin polymerization stimulated by Arp2/3 and the WASP VCA domain. Actin polymerization Biochem Kit™ was used to study the effects of Arp2/3 (Cat. # RP01) and the WASP VCA (Cat. # VCG03) domain on actin polymerization *in vitro*. Arp2/3 or the WASP VCA domain alone has little effect on the rate of actin polymerization while the combination of the two leads to an activation of the actin nucleating Arp2/3 complex and a subsequent increased rate of actin polymerization.

Selected product citations for BK003:
Blader *et al.* 1999, *Mol. Biol. Cell*, 10, 581-596
Fontao *et al.* 2001, *J. Cell Sci.*, 114, 2065-2076
Kumar *et al.* 2004, *J. Biol. Chem.*, 279, 45036-45046
Takamiya *et al.* 2005, *Am. J. Physiol.*, 288, C253-259

G-actin / F-actin *In Vivo* Assay Kit (BK037)

This is a powerful assay that allows for the quantitation of the monomer actin to polymer actin ratio in cells. It also allows the characterization of proteins or compounds that affect *in vivo* actin dynamics.

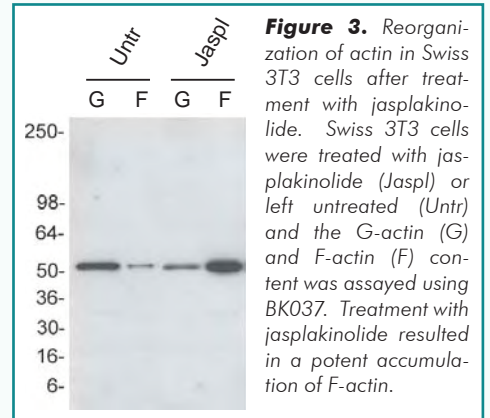


Figure 3. Reorganization of actin in Swiss 3T3 cells after treatment with jasplakinolide. Swiss 3T3 cells were treated with jasplakinolide (Jaspl) or left untreated (Untr) and the G-actin (G) and F-actin (F) content was assayed using BK037. Treatment with jasplakinolide resulted in a potent accumulation of F-actin.

Selected product citations for BK037:
Tu *et al.* 2003, *Cell*, 113, 37-47
Tang *et al.* 2003 *Hypertension*. 42, 858-863

F-actin Visualization Biochem Kit™ (BK005)

The kit allows researchers to fix and permeabilize tissue culture cells while preserving structure of the actin cytoskeleton. Subsequently the actin cytoskeleton is stained with fluorescent (rhodamine) phalloidin that is also provided in the kit.

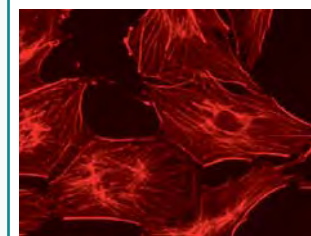


Figure 4. The actin cytoskeleton of Swiss 3T3 cells stained with the F-actin Visualization Biochem Kit™

Actin Biochem Kits™	Cat. #	Amount
Actin Binding Protein Spin-Down Assay Kit™ Characterization of muscle actin binding proteins	BK001	30-100 assays
Actin Binding Protein Spin-Down Assay Kit™ Characterization of non-muscle actin binding proteins	BK013	30-100 assays
Actin Filament Biochem Kit™ For the formation of actin filament substrates	BK002	30-100 assays
Actin Polymerization Biochem Kit™ Pyrene modified actin is used to follow <i>in vitro</i> polymerization	BK003	30-100 assays
Cytoskeleton Isolation Kit Isolates the cytoskeleton and associated proteins	BK039	30-100 assays
F-actin Visualization Biochem Kit™ Stains F-actin networks in fixed cells	BK005	300 assays
G-actin / F-actin <i>In Vivo</i> Assay Kit™ Quantitates <i>in vivo</i> ratio of actin polymer and monomer	BK037	30-100 assays

Actin Filament Biochem Kit™ (BK002)

This kit contains purified actin plus all buffers and reagents necessary to produce actin microfilaments *in vitro*. It is a simple introduction to working with actin *in vitro* and is useful to researchers who simply require actin filament substrates.

Uses for actin filaments include:

- Identification of actin binding proteins (ABPs)
- Characterization of ABPs

Tubulin Biochem Kits™

Absorbance Based Tubulin Polymerization Assay Kits (BK006 & BK004)

Tubulin polymerizes to form microtubules in a dynamic process that is important for many aspects of cell regulation, division and motility. Tubulin dynamics (polymerization versus depolymerization rates) during cell division are also a major target for drug discovery. Polymerization kinetics can be measured by spectroscopic absorbance at 340 nm. Kits BK006 and BK004 provide all reagents and detailed instructions necessary for the performance of polymerization assays.

Kit BK006 uses >99% pure tubulin for polymerization measurements and generates a highly robust signal (Figure 1). BK006 is recommended for the study and characterization of microtubule associated proteins (MAPs) and measurement of IC50 and EC50 values for MAPs and tubulin binding compounds.

Kit BK004 uses >97% tubulin for polymerization measurements. The signal generated by BK004 is approximately 50% that of BK006. This kit is recommended as a cost effective method for preliminary screening and characterization of tubulin binding compounds. See also CytoDYNAMIX Screens CDS01 & CDS03 pages 14-15.

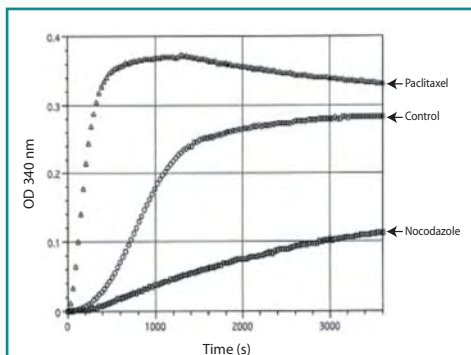


Figure 1. Tubulin polymerization curves. The figure shows a standard polymerization curve for kit BK006 (Control curve). The reaction contains 100 μ l volume of 3 mg/ml tubulin in 80 mM PIPES pH 7.0, 0.5 mM EGTA, 2 mM MgCl₂, 1 mM GTP and 10% glycerol. Polymerization was started by incubation at 37°C and followed by absorption readings at 340 nm. Under these conditions, polymerization V_{max} is enhanced 4 fold in the presence of 10 μ M paclitaxel and reduced 5.5 fold in the presence of 10 μ M nocodazole.

Selected product citations

Chen *et al.* 2005, Mol. Cancer Ther., 4, 562-568
 Gumireddy *et al.* 2005, Cancer Cell, 7, 275-286
 Mooberry *et al.* 1999, Cancer Res. 59, 653-660
 Nair *et al.* 2004, J. Biol. Chem. 279, 41240 - 41248
 Rouzier *et al.* 2005, Proc. Natl. Acad. Sci. U.S.A. 102, 8315-8320

Fluorescence Based Tubulin Polymerization & Depolymerization Assays (BK011 & BK012)

This assay (BK011) measures tubulin polymerization as a function of enhanced fluorescence of a reporter molecule during the polymerization process. An assay optimized for monitoring microtubule depolymerization is also available (BK012). The great advantage of these assays are that they allow miniaturization of the reaction, something that is not easily achievable with the traditional absorbance based assay. The assays are recommended in situations utilizing purified cancer cell line or fungal tubulins where tubulin protein is a limiting factor or in high throughput screens (See HTS section pages 14-15).

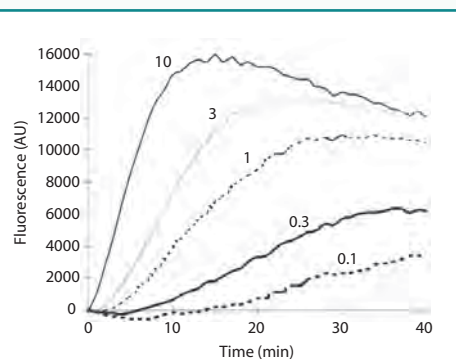


Figure 2. Fluorescence based tubulin polymerization. Tubulin at 2 mg/ml was polymerized using kit BK011 in a total reaction volume of 50 μ l in buffer containing 20% glycerol. Measurements were taken using a 360 nm excitation and 420-450 nm emission filter. The figure shows the enhancement of polymerization as seen by fluorescent signal in response to increasing paclitaxel concentrations (0.1 μ M, 0.3 μ M, 1 μ M, 3 μ M and 10 μ M paclitaxel respectively).

Kits For Producing Microtubules

Fluorescent Microtubules Biochem Kit™ (BK007R)

This kit contains tubulin that has been covalently modified with rhodamine plus all reagents and buffers required for the formation of stable fluorescent microtubules. Rhodamine labeled microtubules are useful substrates for *in vitro* assays such as the kinesin motility assay (Cat. # BK027).

Microtubules / Tubulin Biochem Kit (BK015)

This kit provides purified bovine brain tubulin plus all reagents and instructions necessary for the polymerization of tubulin to form microtubules. These microtubule substrates are useful in the study of Microtubule Associated Proteins (MAPs).

Stable, lyophilized, pre-formed microtubules are also available (Cat. # MT001), see page 21.

Microtubule Binding Protein Spin-Down Assay Kit (BK029)

The *in vitro* interaction of MAPs with microtubules can be studied using this kit.

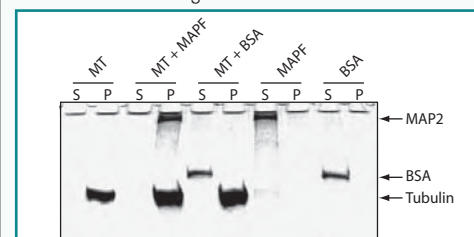


Figure 3. MAP Spin-Down Assay using BK029. Microtubules were mixed with buffer (MT), MAP proteins (MT + MAPF) or BSA (MT + BSA) and MTs were pelleted by centrifugation at 100,000 x g. Supernatant (S) and Pellet (P) fractions were examined by SDS-PAGE. MAP proteins, but not BSA, bind to MTs and co-precipitate. MAP (MAPF) or BSA (BSA) proteins alone do not pellet.

Tubulin Biochem Kits™	Cat. #	Amount
Tubulin Polymerization Assay Kit (>97% pure tubulin) Detects compounds that affect tubulin polymerization	BK004 (CDS01-A)	24-30 assays
Tubulin Polymerization Assay Kit (>99% pure tubulin) Detects compounds that affect tubulin polymerization	BK006 (CDS03-A)	24-30 assays
Fluorescent Microtubules Biochem Kit (rhodamine) Production of fluorescent microtubules	BK007R	50-200 assays
Tubulin Polymerization Assay (fluorescence based) Detects compounds that affect tubulin polymerization	BK011	96 assays
Microtubule Stabilization / Destabilization Assay Detects compounds that affect tubulin depolymerization	BK012	96 assays
Microtubules / Tubulin Biochem Kit Production of microtubules	BK015	8-200 assays
Microtubule Binding Protein Spin-Down Assay Kit Characterization of microtubule binding proteins	BK029	30-100 assays
Microtubule / Tubulin In Vivo Assay Kit Quantitates <i>in vivo</i> ratio of tubulin polymer & monomer	BK038	30-100 assays
Cytoskeleton Isolation Kit Isolates the cytoskeleton and associated proteins	BK039	30-100 assays

ATPase & GTPase Biochem Kits™

ATPase, GTPase and Phosphatase Biochem Kits™

ATPases, GTPases and other phosphatases liberate inorganic phosphate (Pi) from their respective triphosphate nucleotide or substrate. This fact can be used to measure their activity. Cytoskeleton, Inc. has introduced the largest range of phosphate assays currently available. They allow the researcher to choose an exact fit for their application. The assays are all highly robust. BK050-BK055 and BK105 are all suitable for HTS applications and our experienced technical assistance staff can help you incorporate these assays into your HTS facility.

Biochem Kits™ BK050-BK054 and BK060 measure liberated phosphate via binding to a reporter dye or by enzymatic conversion into a reporter molecule, whereas BK055 measures liberated radioactive phosphate.

BK053 and BK054 are end point assays suitable for activity assays such as microtubule induced kinesin ATPase or F-actin induced myosin ATPase.

BK055 was specifically introduced for low activity enzymes (Kcat <0.01), which usually turn

over their substrate only once or very slowly such as small G-proteins, tubulin or actin.

BK051, BK052 and BK060 are kinetic assays and are therefore suitable for Vmax or Kcat determinations. These kits require a higher level activity ATPase or GTPase for sufficient sensitivity.

BK105 is specifically developed to detect the low Pi production by small G-proteins in an end point non-radio-active assay.

See table below for details about the assays.

Kit	Type of assay	P _i Detection limit	Uses	Color change	# of steps	Strengths
BK050 PhosFree™ Phosphate Assay Kit	Endpoint Colorimetric	1 μM (0.1 nmol)	HA, HP, HTS, MA	Yellow to Green	2	Measure Pi in high protein concentrations
BK053 Kinesin ATPase End Point Assay	Endpoint Colorimetric	1 μM (0.1 nmol)	HA, HTS, MA, MP	Yellow to Green	3	HTS compatible
BK054 CytoPhos™ Phosphate Assay	Endpoint Colorimetric	1 μM (0.1 nmol)	HA, HTS, MA	Yellow to Green	2	HTS compatible
BK055 EasyRad™ Phosphate Assay	Endpoint Radioactive	20 nM (2 pmol)	AT, HG, HTS, LA, SG	N/A	2	Very low detection limit
BK051/52 ATPase/GTPase ELIPA™	Kinetic Colorimetric	7 μM (2 nmol)	HA	Non-visible (360 nM)	Homogeneous	Kinetic
BK060 Kinesin ELIPA™ Biochem Kit	Kinetic Colorimetric	7 μM (2 nmol)	HA, MP	Non-visible (360 nM)	Homogeneous	Kinetic

HA = High activity (Kcat >0.1). MA = Medium activity (Kcat 0.01 to 0.1). LA = Low activity (Kcat <0.01). HP = High protein content in reaction (>0.5 mg/ml). HTS = High throughput compatible. HG = Heterotrimeric G-proteins. SG = Small G-proteins. MP = Motor proteins. AT = Actin and tubulin ATPase and GTPase measurements.

The Phosphate Assay Principle

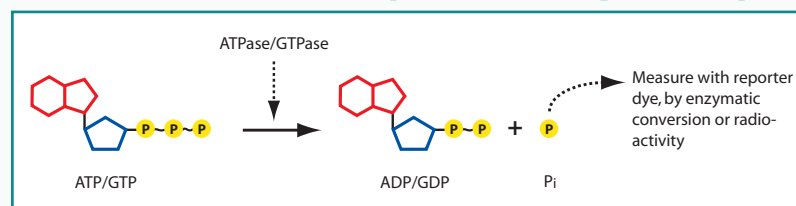


Figure 1. The principle of using a phosphate assay to measure activity of ATPases and GTPases. Pi generation can also be used to measure the activity of other phosphatases.

P_i Standard Curves

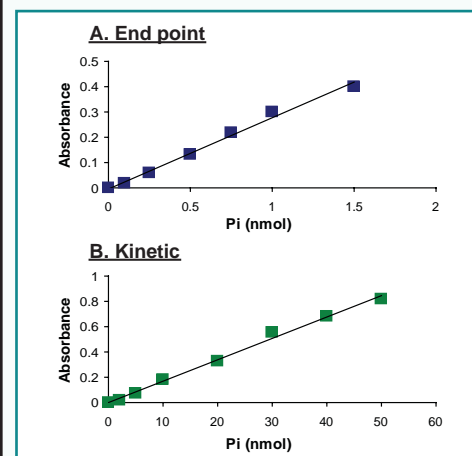


Figure 2. Comparison of standard curves of Cytoskeleton's end point (BK050, BK053 and BK054) and kinetic (BK051/52 and BK060) phosphate assays. End point assays have a linear response between 0.1 and 1.5 nmol Pi. Kinetic assays give a linear response between 2 and 50 nmol Pi.

Phosphate Quantitation Biochem Kits™

	Cat. #	Amount
ATPase ELIPA™ (enzyme linked, absorbance) Kinetic quantitation of ATP hydrolysis	BK051	96 assays
CytoPhos™ Phosphate Assay (end point assay) Absorbance assay for measuring activity of ATPases & GTPases	BK054	1000 assays
EasyRad™ Phosphate Assay Simple assay for radioactive detection of GTPase activity (radioactive nucleotides not supplied) (Kcat >0.0001)	BK055	1000 assays
GAP Assay Biochem Kit Measures GTPase activity of small G-proteins	BK105	80-160 assays
GTPase ELIPA™ (enzyme linked, absorbance) Kinetic quantitation of GTP hydrolysis	BK052	96 assays
Kinesin ELIPA™ Biochem Kit For real time kinetic and Vmax kinesin ATPase measurements	BK060	96 assays
Kinesin ATPase End Point Assay For end point measurement of kinesin ATPase activity	BK053	1000 assays
PhosFree™ Phosphate Assay Kit Colorimetric assay for high protein concentrations	BK050	1000 assays

Intermediate Filament Biochem Kits™

	Cat. #	Amount
Vimentin Biochem Kit Production of vimentin filaments <i>in vitro</i>	BK020	5-50 assays
Vimentin Protein Recombinant	V01-A V01-C	2 x 50 μg 10 x 50 μg
Cytoskeleton Isolation Kit Isolates the cytoskeleton and associated proteins	BK039	30-100 assays

Vimentin Biochem Kit™

This kit allows the user to work with vimentin in the tetrameric (unpolymerized form) and/or the polymeric filamentous form. Cat. # BK020. Uses include:

- Investigating vimentin filament dynamics.
- Identification of vimentin associated proteins.
- Discovery of intermediate filament associated drugs.

Signal Transduction Biochem Kits™

G-LISA™ - A Revolutionary Way of Doing Small G-protein Activation Assays!

Measuring the activation (i.e. the level of GTP-loading) of small GTPases in cells or tissues has become a widely used technique for signal transduction researchers in recent years.

Traditionally, this has been done by pulldown methods. While these assays have proven very useful, they have many drawbacks such as being time consuming, requiring large amounts of cell material, being limited in the number of samples that can be handled simultaneously and yielding only semi-quantitative results. To address this, Cytoskeleton, Inc has developed the revolutionary G-LISA™ assay.

The G-LISA™ Advantage

With the new G-LISA™ kits for Rho family proteins (patent pending) you can now measure RhoA, RhoA,B,C, Rac1 or Rac1,2,3 activity from cell and tissue samples in less than 3 h. The kits are available in either absorbance or luminescence detection formats (see product table on p.11). G-LISA™ requires only 1-5% of the cell material needed for a conventional pulldown assay, and the assay is so simple that it allows you to handle much larger sample numbers than you could do before. As opposed to pulldown assays, where the output is a western blot, G-LISA™ gives you numerical data which is easily compared between samples. See Table 1 below for a summary of the other G-LISA™ advantages.

New This Year !

The Rac1,2,3 (BK125) and Rac1 specific (BK126) versions are new this year. They are very robust and accurate assays that generate data five times faster than conventional formats. In Figure 2, the signal to noise ratio is shown from HeLa and Swiss 3T3 cell extracts. In Figure 3, the coefficient of variation is compared with the sensitivity to different concentrations of cell lysate protein.

Drug Discovery

See page 17 for more information on using G-LISA™ in drug discovery formats. If you are considering high throughput screens using the G-LISA™ kit, please inquire for significant discounts.

The G-LISA Method

The G-LISA™ assay is based on using a 96-well plate where a Rho- or Rac-GTP-binding domain has been covalently coupled to the wells. Active, GTP-bound Rho or Rac in lysates will bind to the wells while inactive, GDP-bound protein, is washed away. The bound active Rho or Rac protein is subsequently detected with a specific antibody and chemiluminescence or absorbance.

Since the Rho- or Rac-GTP affinity wells are supplied as strips and the strips can be broken into smaller pieces, each kit can be used for anywhere from 2 to 96 assays.

For a brief overview of the method, see Figure 1 below.

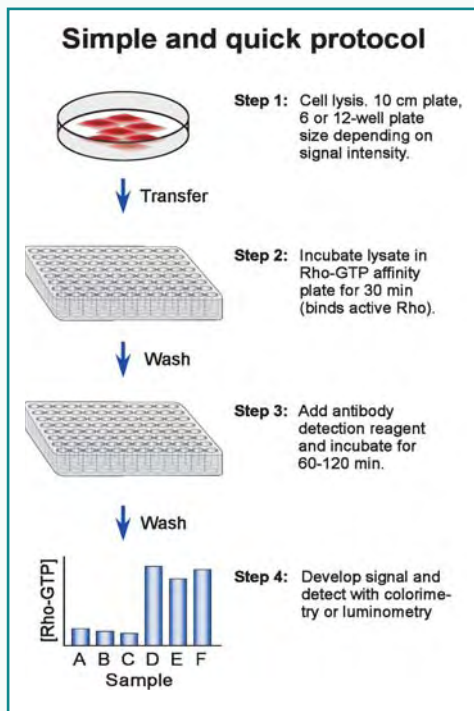


Figure 1. A schematic description of the G-LISA™ assay.

Rac G-LISA™ Results

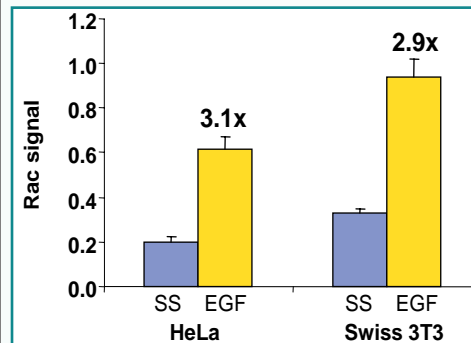


Figure 2. Rac activation of HeLa and 3T3 cells by EGF measured by Rac G-LISA™. Cells were serum starved (SS) for 24 h and treated with EGF (10 ng/ml for 2 min). 20 µg of lysates were subject to the G-LISA™ assay. Absorbance was read at 490 nm.

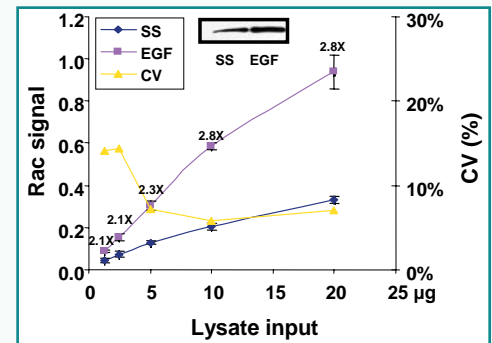


Figure 3. Rac activation by EGF measured by the G-LISA™ kit BK125. Swiss 3T3 were serum starved (SS) for 24 h followed by stimulation with EGF (10 ng/ml for 2 min). 20, 10, 5, 2.5 µg of lysates were subjected to the G-LISA™ assay. Absorbance was read at 490 nm. 500 µg of the same lysates were subject to the traditional PAK pulldown assay (shown in inset).

G-LISA™ vs Pulldown Activation Assays

While G-LISA™ assays represent a novel way of testing the activation levels of small G-proteins, the results that you get are comparable to ones that a pulldown assay would yield. Therefore, you can comfortably switch from a pulldown approach to G-LISA™ and still be able to compare your new results with previously generated data.

The big advantages in using G-LISA™ over conventional pulldowns are the time saved, the fact that you can do more assays simultaneously, that you get quantitative data without having to run a PAGE gel and scan X-ray films, and that the amount of cell material needed for each assay is significantly reduced. If you are working with precious material such as primary cells, using G-LISA™ will allow you to test Rho activation much more economically than before.

Due to the strong interspecies conservation of Rho, G-LISA™ assays are likely to work in most species. Check the website for a list of species and cell types that have been tested.

Table 1: Comparison of conventional Rho pulldown assay to G-LISA™

	Conventional pulldown	G-LISA™
Assay Time	10-12 h (2 days)	<3 h
Cell material per assay	0.5-1 mg protein	10-50 µg protein
Sample handling	Up to 10 samples	96 samples or more
Quantitative data	Semi	Yes
HTS compatible	No	Yes

Signal Transduction Biochem Kits™

GEF Assay

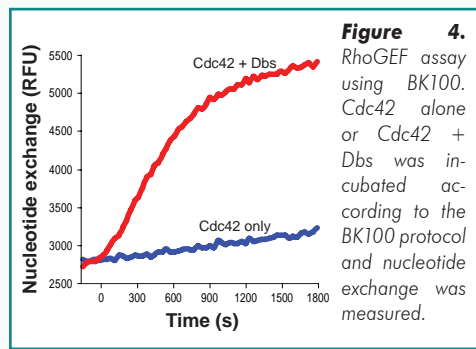
GEFs function by catalyzing the exchange of nucleotide on their target G-proteins, leading to a switch from a GDP-bound, inactive state, to GTP-bound, active, state.

Cytoskeleton's GEF Assay Kit (Cat. # BK100) is a fluorophore based assay for measuring nucleotide exchange on GTPases in either 96-well or 384-well format. The kit measures the uptake of a fluorescent nucleotide analog into GTPases.

The GEF assay kit contains all the buffers and reagents needed for the assay, including Cdc42, Rac1 and RhoA GTPase proteins and the GEF domain of Dbs as a positive control GEF. While the kit comes with Cdc42, Rac1 and RhoA proteins, it can also be used for any other small G-protein. See page 18 for purified GTPases available from Cytoskeleton, Inc.

Uses of the GEF assay kit include:

- Determine the activity and specificity of uncharacterized GEFs
- Regulation of GEF activity by cofactors.
- Discovery of small molecule GEF inhibitors



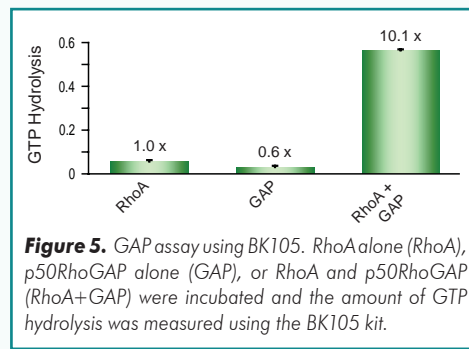
GAP Assay

Small G-proteins typically have an intrinsic GTPase activity, meaning that after they get activated (GTP-bound), they "turn themselves off" by hydrolysis of the bound GTP to GDP. Their GTPase rate, however, is normally slow. So GAPs, which act by stimulating the G-protein's intrinsic GTPase activity, are important signaling molecules that function to terminate the G-protein signals.

Cytoskeleton's GAP assay kit (Cat. # BK105) measures the amount of inorganic phosphate (P_i) that is produced as a result of G-protein dependent hydrolysis of GTP to GDP + P_i in a 96-well or 384-well format. The kit contains all the reagents needed for the assay including Cdc42, Rac1, RhoA and H-Ras GTPases, a positive control RhoGAP, carefully optimized buffers and phosphate detection reagent.

Uses of the GAP assay kit include:

- Determine the activity and specificity of uncharacterized GAPs
- Regulation of GAP activity by cofactors.
- Discovery of small molecule GAP inhibitors

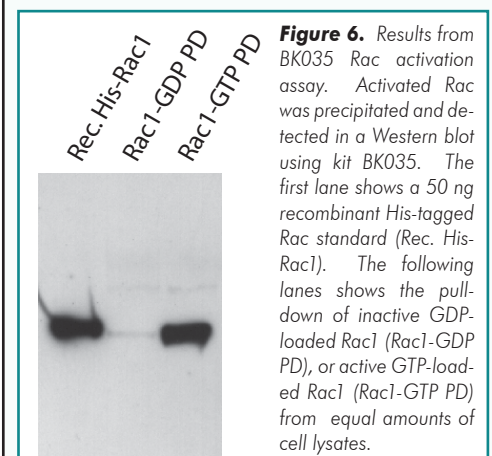


Pulldown Activation Assays

As a complement to our G-LISA™ assays we also provide the traditional pulldown assays for measuring activation levels of Ras, RhoA, Rac and Cdc42 from cells and tissues.

These assays work by using a domain of a G-protein effector coupled to glutathione beads. These affinity beads only bind the active, GTP-bound, form of the target G-protein, thereby isolating the active pool of the target G-protein from a cell lysate. The precipitated material is run on an SDS-PAGE gel followed by a Western blot for the G-protein to measure the amount of active G-protein.

The kits come with all reagents needed for the assay, including colored activated G-protein affinity beads (which are easy to see, Fig.7) and a specific antibody. (see BK008, BK034, BK035, BK036).

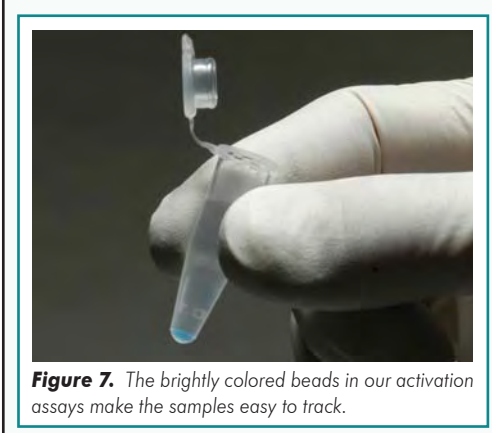


G-LISA™ Kits

	Cat. #	Amount
NEW G-LISA™ for Rac1, luminescence based Quantitates <i>in vivo</i> activation of Rac1 only	BK126	96 assays
NEW G-LISA™ for Rac1,2 and 3, colorimetric based Quantitates <i>in vivo</i> activation of Rac1,2,3 combined	BK125	96 assays
G-LISA™ for RhoA, luminescence based Quantitates <i>in vivo</i> activation of RhoA only	BK121	96 assays
G-LISA™ for RhoA,B,C, colorimetric based Quantitates <i>in vivo</i> activation of RhoA,B and C combined	BK123	96 assays
G-LISA™ for RhoA, colorimetric based Quantitates <i>in vivo</i> activation of RhoA,B,C	BK124	96 assays

Other Signal Transduction Biochem Kits™

	Cat. #	Amount
GEF Assay Biochem Kit™ Based on Mant-GTP. Exchange increases fluorescence	BK100	60-300 assays
GAP Assay Biochem Kit™ Measures GAP enhanced GTPase activity	BK105	80-160 assays
RhoA Activation Assay Biochem Kit™ Pull-down assay to measure <i>in vivo</i> activation of RhoA	BK036	30-60 assays
Cdc42 Activation Assay Biochem Kit™ Pull-down assay to measure <i>in vivo</i> activation of Cdc42	BK034	25-50 assays
Rac Activation Assay Biochem Kit™ Pull-down assay to measure <i>in vivo</i> activation of Rac1	BK035	25-50 assays
Ras Activation Assay Biochem Kit™ Pull-down assay to measure <i>in vivo</i> activation of Ras	BK008	25-50 assays



Small G-protein High Throughput Assays

Several of Cytoskeleton, Inc's G-protein Biochem Kits™ are adaptable for high throughput format. These include the GEF, GAP and G-LISA™ assays. Please inquire to tservice@cytoskeleton.com for more information.

Cytoskeleton Motor Werks™

About Cytoskeleton Motor Werks™ Products

The Cytoskeleton Motor Werks™ (CMW) line of products focuses on the kinesin family of eukaryotic motor proteins. The product line includes highly pure and biologically active recombinant motor domain proteins (see below). These are useful reagents for anti-mitotic drug discovery and the mechanistic study of kinesin activity (see description below and Table: Purified Motor Proteins).

The Cytoskeleton Motor Werks™ line also contains several Biochem Kits™ that are designed to aid in kinesin research:

Measurement of Microtubule Activated Kinesin ATPase Activity: The fact that kinesin ATPase activity is greatly enhanced upon microtubule binding provides a robust assay for measuring kinesin motor activity. There are two kinesin ATPase assays available, both are absorbance based and monitor the release of inorganic phosphate (P_i). BK060 is a kinetic enzyme linked inorganic phos-

phate assay (ELIPA™) and has a detection limit of 7 μM P_i. BK053 is a rapid, homogenous end point assay with a detection limit of 1 μM P_i (Funk *et al.* 2004, *Anal. Biochem.* 329, 68-76). Both of these assays are supplied with pre-formed microtubules (Cat. # MT001, see pages 13 & 21). Uses include:

- Kinesin Vmax determinations (BK060)
- HTS screens for kinesin inhibitors (BK053)
- Kinesin inhibitor IC50 determinations (BK060)

MCAK Microtubule Depolymerization Assay: MCAK belongs to the mitotic class of motors that have microtubule depolymerizing activity. We have developed a fluorescence based microtubule depolymerization assay (BK012) that can be used for:

- Measuring MCAK activity *in vitro*
- Discovery of inhibitors of MT depolymerizing kinesins.

New This Year

Cardiac Myosin (Cat.# MY03) for F-actin stimulated ATPase assays and Pre-formed F-actin filaments (Cat.# AKF99) which are user-ready for pulldown assays or ATPase assays.

Kinesin Motility Assays: The motor domain of kinesin proteins are able to bind to microtubules and utilize the energy of ATP hydrolysis to translocate along the microtubule. This property is a key characteristic of most kinesins and is essential to their role in intracellular transport. BK027 contains all the necessary components to perform this assay in fluorescence or DIC formats. Technical assistance for setting up this assay is available at tservice@cytoskeleton.com. Uses include:

- Monitoring kinesin motor activity
- Screening for kinesin inhibitors

Purified, Biologically Active Kinesin Motor Domain Proteins

There are approximately 50 human kinesins that are currently divided into at least 14 classes. Kinesins are involved in almost all aspects of intracellular transport and their well documented role in cell division suggests that they may be excellent targets for anti-mitotic drug discovery. All kinesin proteins have a conserved motor domain that contains a microtubule binding site and the ATP binding / hydrolysis site. The motor domain of various kinesins have widely differing sensitivities to ATP analogs and inhibitory compounds such as monastrol. Such results lead the way to the identification of therapeutically useful kinesin specific inhibitors.

Cytoskeleton, Inc. has made available a selection of recombinant human kinesin motor domain proteins from 9 of the 14 reported kinesin classes. The proteins are extensively quality controlled for purity (>85%) and biological activity (microtubule activated ATPase activity comparable to published data). The protein domains are useful as targets for anti-mitotic drug discovery and as reagents for comparative studies of kinesin class specific motor activities. Examples of kinesin purities are shown below.

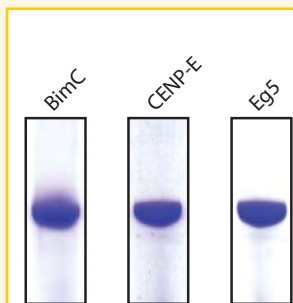


Figure 1. Purity of kinesin motor domain proteins. BimC (BM01), CENP-E (CP01) and Eg5 (EG01) were run on SDS-PAGE gels and stained with coomassie blue.

Myosin Products

In addition to kinesin products, the Cytoskeleton Motor Werks™ line also includes myosin products.

Cytoskeleton, Inc provides purified Cardiac Myosin from bovine heart (Cat. # MY03) and Myosin II from rabbit skeletal muscle (Cat. # MY02). The preparations contain the regulatory light chains and they are ideal for F-actin stimulated ATPase assays. Cytoskeleton also provides heavy meromyosin (Cat. # MH01), a proteolytic fragment of Myosin

II consisting of the two head domains connected by the subfragment-2 regions. This product is also biologically active and its ATPase activity is greatly enhanced in the presence of actin filaments.

Myosin ATPase activity can be tested using the kinetic ATPase ELIPA™ Biochem Kit™ (Cat. # BK051) or the end point CytoPhos™ Phosphate Assay (Cat. # BK054). For more information on these assays, turn to page 9.

Purified Motor Proteins	Cat #	Amount
CENP-E Motor Domain Protein (<i>H. sapiens</i>)	CP01-A	2 x 25 μg
	CP01-B	10 x 25 μg
Chromokinesin Motor Domain Protein (<i>H. sapiens</i>)	CR01-A	2 x 25 μg
	CR01-B	10 x 25 μg
Eg5 Motor Domain Protein (<i>H. sapiens</i>)	EG01-A	2 x 25 μg
	EG01-B	10 x 25 μg
Eg5 homolog BimC Motor Domain Protein (<i>A. nidulans</i>)	BM01-A	2 x 25 μg
	BM01-B	10 x 25 μg
Eg5 Homolog BimC Motor Domain Protein (<i>A. fumigatus</i>)	EG02-A	2 x 15 μg
	EG02-B	10 x 15 μg
KIFC3 Motor Domain Protein (<i>H. sapiens</i>)	KC01-A	2 x 25 μg
	KC01-B	10 x 25 μg
KIF3C Motor Domain Protein (<i>H. sapiens</i>)	KF01-A	2 x 25 μg
	KF01-B	10 x 25 μg
Kinesin Heavy Chain Motor Domain Protein (<i>H. sapiens</i>)	KR01-A	2 x 25 μg
	KR01-B	10 x 25 μg
MCAK Motor Domain Protein (<i>H. sapiens</i>)	MK01-A	2 x 25 μg
	MK01-B	10 x 25 μg
MKLP Motor Domain Protein (<i>H. sapiens</i>)	MP01-A	2 x 25 μg
	MP01-B	10 x 25 μg
Myosin II Skeletal Muscle Protein (rabbit)	MY02-A	5 x 1 mg
	MY02-B	20 x 1 mg
NEW Myosin, Cardiac Muscle Protein (bovine)	MY03-A	1 x 1 mg
	MY03-B	5 x 1 mg
Heavy Meromyosin Skeletal Muscle Protein (rabbit)	MH01-A	4 x 50 μg
	MH01-B	20 x 50 μg

NOTE: For bulk amounts of these motors, see page 16.

Cytoskeleton Motor Werks™

Kinesin Antibodies

Cytoskeleton Inc. has generated a set of polyclonal antibodies that are ideal for a range of applications in kinesin research. In particular, the antibodies have been tested for immunoprecipitation, immunocytochemistry, Western blot analysis, inhibition of target kinesin ATPase activity and species cross reactivity.

All of Cytoskeleton's kinesin antibodies are excellent for use in Western blots and immunocytochemistry. Most of them also work for immunoprecipitation, allowing the user to identify associated proteins and to concentrate the motor protein for

applications such as motility assays (Field *et al.* 1998, *Methods Enzymol.* 298, 525-541). Several of the antibodies can also inhibit the ATPase activity of the antigen.

Included with each antibody is a native protein cell extract which has been developed for the sole purpose of providing a rigid quality control check on the antibody's activity.

Please ask our technical assistance (tservice@cytoskeleton.com) to email you a datasheet so that you can find out the specifics of the antibody you are interested in before purchasing.

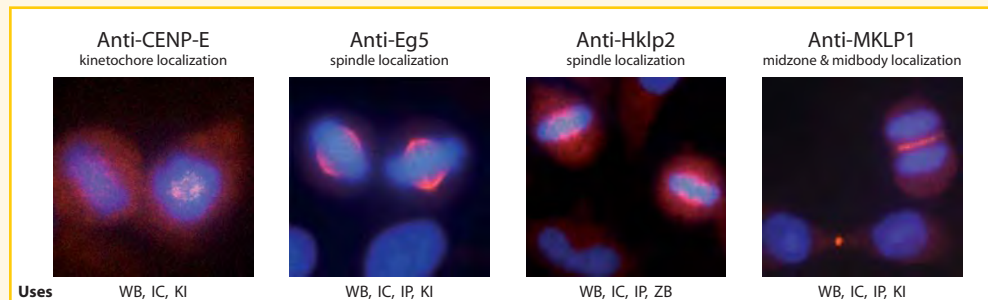


Figure 2. Examples of immunocytochemistry stainings with Cytoskeleton, Inc's kinesin antibodies. HeLa cells were stained with each specific antibody and DAPI. Uses that the antibodies have been

tested for are listed under the images.

WB, western blot; IC, immunocytochemistry; IP, immunoprecipitation; KI, kinesin ATPase inhibitory activity; ZB, zoo blot - species cross reactivity.

Kinesin Biochem Kits™

Cytoskeleton Inc provides the widest range available of kits to assay kinesins. These assays include kinetic and endpoint ATPase assays for kinesins as well as kinesin motility assays and microtubule depolymerization assays for depolymerizing kinesins such as MCAK. For more information on these kits, see pages 9 and 16.

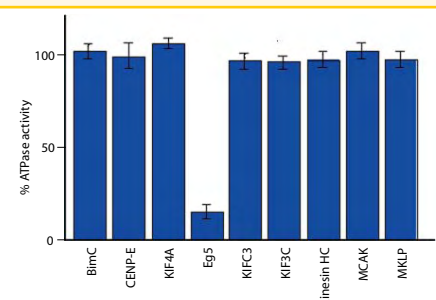


Figure 3. Inhibition of kinesin activity with the Eg5 specific inhibitor monastrol. The results show the percent of full kinesin activity in the presence of 100 μM monastrol. As expected, only Eg5 ATPase activity is inhibited by monastrol.

Pre-formed Microtubules

Microtubule (MT) substrates are required for the study of the activity of kinesin motors. Scientists at Cytoskeleton, Inc. have developed a patented method for MT production and lyophilization that ensures highly reproducible MTs (2 μm average length). Pre-formed microtubules (Cat. # MT001) provide an extremely convenient way to perform assays requiring microtubules as a substrate. Microtubules are supplied as a stable lyophilized powder, simply resuspend and use. Uses for pre-formed stabilized microtubules include:

- Substrate for activation of kinesin ATPase activity
- Determination of IC50s for kinesin inhibitors
- Ideal for HTS applications (inquire for bulk rates)

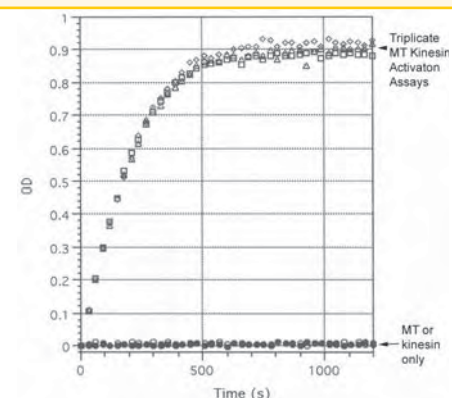


Figure 4. Microtubule activated kinesin ATPase assay using MT001. Each condition was performed in triplicate.

Motor Protein Antibodies

	Cat #	Amount
Kinesin Heavy Chain Antibody (Drosophila antigen)	AKIN01-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN01-B	3 x 50 μg
Eg5 Antibody (Human motor domain antigen)	AKIN03-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN03-B	3 x 50 μg
CENP-E Antibody (Human motor domain antigen)	AKIN04-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN04-B	3 x 50 μg
MCAK Antibody (Human motor domain antigen)	AKIN05-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN05-B	3 x 50 μg
MKLP1 Antibody (Human motor domain antigen)	AKIN06-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN06-B	3 x 50 μg
KIF3C Antibody (Human stalk region antigen)	AKIN09-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN09-B	3 x 50 μg
KIF1C Antibody (Human stalk region antigen)	AKIN11-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN11-B	3 x 50 μg
Kid Antibody (Human stalk region antigen)	AKIN12-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN12-B	3 x 50 μg
Hklp2 Antibody (Human stalk region antigen)	AKIN13-A	1 x 50 μg
Affinity purified rabbit polyclonal, includes positive control	AKIN13-B	3 x 50 μg

Other Motor Protein Reagents

	Cat #	Amount
Microtubules, Pre-formed, lyophilized	MT001-A	4 x 500 μg
A ready to use substrate for motor ATPase assays	MT001-XL	1 x 10 mg
Actin Filaments, Pre-formed, lyophilized	AKF99-A	1 x 1 mg
A ready to use substrate for myosin ATPase assays	AKF99-B	5 x 1 mg
Paclitaxel (2 mM stock)	TXD01	10 x 100 μl
Stabilizes microtubules in motor assays		
Kinesin/Dynein Motility Kit	BK027	25 assays
Measures <i>in vitro</i> motor protein velocities		
Perfusion Chambers	BSM05-04	25 chambers
Chambers used in motility assays		

Drug Target: Tubulin

Tubulin Protein

Tubulin is available in >99% purified, MAP-depleted (>97% pure) and MAP-enriched varieties. See pages 20-21 for more information on our full line of tubulin proteins.

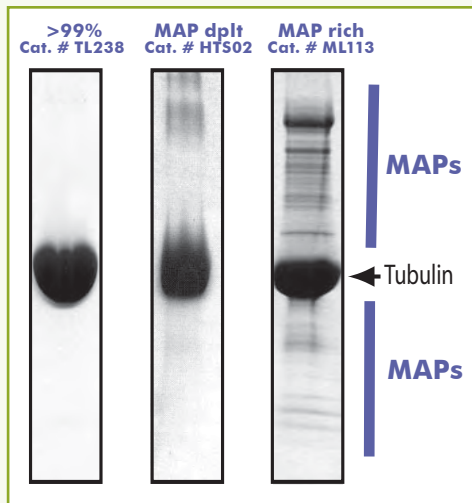


Figure 1. Tubulin purity. 100 µg of >99% pure tubulin (TL238), MAP-depleted (HTS02) and MAP enriched tubulin (ML113) were run on SDS-PAGE gels and stained with coomassie blue.

IC50 Determination For Vinblastine Using Pure Tubulin

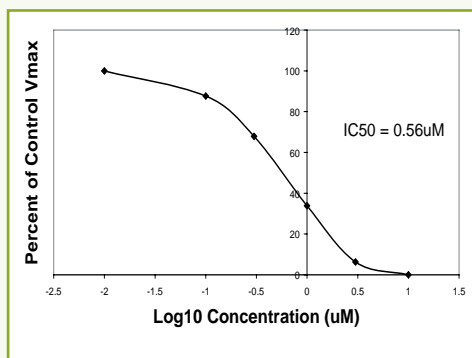


Figure 2. IC50 determination for vinblastine using pure tubulin. Results obtained Pure tubulin was polymerized at 2 mg/ml in G-PEM plus 20% glycerol and polymerization was measured with the fluorescence based tubulin polymerization assay (Cat. # BK011). Compound was added as a 10 x concentrated stock prior to inducing polymerization at 37°C. Data were reduced to Vmax's and the percent of Vmax value was calculated based on 100% being equivalent to the control. This percent Vmax was plotted on the dose-response graph. IC50 for vinblastine was determined to be 0.56 µM.

Tubulin Drug Discovery

Tubulin provided by Cytoskeleton, Inc. has been used as a standard in the research and drug discovery field for over 10 years (see citations below). Polymerization assays offer the most flexible assay system for identifying and measuring tubulin ligands. Both fluorescence and optical density formats are available in 96-well (O.D. or fluo.) or 384-well (fluo.) footprints.

Uses in drug development

- Drug screening for new tubulin ligands
- Negative control for anti-mitotic screen
- IC50 measurements for known drugs
- Lead optimization with SAR

Example Citations

Drug screening: Haggarty, S. J. *et al.* (2003). Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 4389-4394.

Lead optimization: Liou, J. P. *et al.*, (2004). Concise synthesis and structure-activity relationships of combretastatin A-4 analogues, 1-aroylindoles and 3-aroylindoles, as novel classes of potent anti-tubulin agents. *J. Med. Chem.* **47**, 4247-4257.

IC50 measurements: Loganzo, F. *et al.* (2003). HTI-286, a synthetic analogue of the tripeptide hemimasterlin, is a potent antimicrotubule agent that circumvents P-glycoprotein-mediated resistance in vitro and in vivo. *Cancer Res.* **63**, 1838-1845.

Negative control secondary screens: Gummireddy, K. *et al.* (2005). ON01910, a non-ATP-competitive small molecule inhibitor of Plk1, is a potent anticancer agent. *Cancer Cell* **7**, 275-286.

Polymerization assay 96-well optical density format

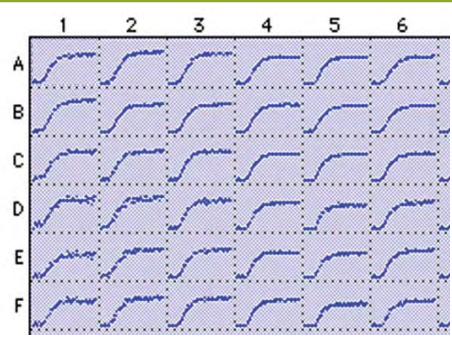


Figure 3. Tubulin polymerization assay in 96-well optical density format. 10 µl of 10 x concentrated compounds are added to each well, followed by 100 µl of tubulin (either HTS02, ML113 or TL238) at 2 to 4 mg/ml. Incubate at 37°C, OD340 nm.

Polymerization assay 384-well fluorescence format

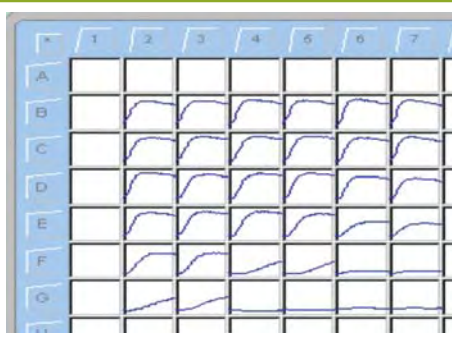


Figure 4. Tubulin polymerization assay in 384-well fluorescence format. 2 µl of 10 x concentrated compound are pipetted into each well, followed by 10 µl of tubulin (H001, H005 or TL238) at 2.4 mg/ml. Incubate at 37°C, Ex 360, Em 410-450 nm.

Description	Cat. #	Amount
Tubulin Polymerization Assay Kit (>99% pure tubulin) Detects compounds that affect tubulin polymerization	CDS03-A	24 assays
	CDS03-B	96 assays
Tubulin Polymerization Assay Kit (>97% pure tubulin) Detects compounds that affect tubulin polymerization	CDS01-A	24 assays
	CDS01-B	96 assays
Tubulin Protein (>99% pure) Bovine brain source	TL238-C	5 x 1 mg
	TL238-E	20 x 1 mg
	TL238-DX	1 x 10 mg
Tubulin Protein (>99% pure) Porcine brain source	T240-B	5 x 1 mg
	T240-C	20 x 1 mg
	T240-DX	1 x 10 mg
Tubulin Protein, MAP-depleted (>97% pure) Bovine brain source, for HTS applications	HTS02-A	1 x 4 mg
	HTS02-B	1 x 40 mg
	HTS02-XL	1 x 100 mg
Tubulin Protein, MAP-enriched Bovine brain source, 70% tubulin, 30% MAPs	ML113-C	5 x 1 mg
	ML113-E	20 x 1 mg
	ML113-DX	1 x 10 mg
Tubulin protein, MAP-enriched Porcine brain source, 70% tubulin, 30% MAPs	ML116-B	5 x 1 mg
	ML116-C	20 x 1 mg
	ML116-DX	1 x 10 mg

Drug Target: Tubulin

Site Specific Competition Assays

Site specific competition assays are valuable tools for screening and identifying new compounds that hit known sites in the tubulin molecule. Examples of these sites are the colchicine, vinblastine and taxol binding sites of tubulin. However, there are a multitude of newly discovered sites (e.g. epothilone and halichondrin binding sites) that can also be probed with the reagents described in this section.

The main assay is based on biotinylated tubulin (Cat. # T333, H003 or H007), which is coupled to SPA beads (GE Healthcare Inc.) as originally described by Tahir *et al.* 2000 (Biotechniques, 29, 156-160).

The key components that Cytoskeleton, Inc. has

developed are biotinylated derivatives of tubulin, which retain high biological activity. A long spacer arm between the biotin and tubulin allows rapid access to all sites on tubulin.

A simple application of tritiated parent site compound to only 1 μg of biotinylated tubulin attached to SPA beads results in a large signal to noise ratio (up to 15:1, see Figure 5). The assays are HTS compatible due to the ease of automation and the low amounts of tubulin required.

New this year are biotinylated derivatives of cancer cell tubulin, which are ideal reagents to target cancer cells specifically (Cat. # H003 and H007).

Tubulin Ligand Competition Assay

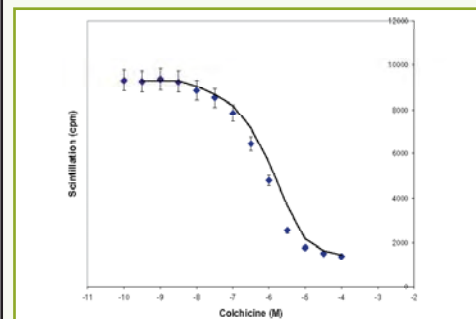


Figure 5. Biotinylated tubulin was incubated with SPA beads (GE Healthcare Inc.) and tritiated colchicine for 30 min, and each well was read for 10 s. Cat.# CDS15.

Description	Cat. #	Amount
Tubulin Ligand Competition Assay SPA based assay, the kit contains biotinylated tubulin (Cat.#T333) and all buffers necessary to perform the assay. In addition, the kit requires purchase of SPA beads, plates and tritiated parent site compound.	CDS15	1000 assays
Tubulin Protein, biotinylated Bovine brain source (58% β II, 25% β III, 14% β IV isotypes)	T333-XL	1 x 500 μg
Tubulin Protein, biotinylated HeLa cancer cell source (90% β I, 10% β IV isotypes)	H003	1 x 40 μg
NEW Tubulin Protein, biotinylated MCF-7 cancer cell source (55% β I, 6% β III, 39% β IV)	H007	1 x 40 μg

Uses in Drug Development

- Site specific tubulin ligand development
- Determine K_i values versus parent drug
- K_d measurements for known drugs
- Lead optimization with SAR
- Compare drug affinity for different isotypes of tubulin

Specialized Tubulins For Improved Disease Targeting

Cytoskeleton is interested in exploiting the diversity between host and pathogen tubulin isotypes to develop better targeted drugs. Here are a few examples that we are currently working on:

The controlled pair of taxol research cell lines (1A9 and PTX22) can be used to isolate pure tubulin which can be used to study the effects of novel drugs on paclitaxel resistant tubulin. 1A9 cell tubulin has parental tubulin composition whereas PTX22 has a specific mutation in β -tubulin A364T (Giannakakou *et al.* 1997, J. Biol. Chem., 272, 17118-25) making it paclitaxel resistant. These tubulins can be used in the fluorescent polymerization or SPA formats, bringing them into the realms of drug screening feasibility.

Another approach to studying taxol resistance is to measure the potency of taxol analogs or other

ligands on their ability to affect or bind tubulin that is enriched in the β III isotype. This isotype has been shown to be a cause of taxol insensitivity *in vitro* (Ferlini *et al.* 2005, Cancer Res., 65, 2397-2405) and its expression is correlated with poor prognosis in taxol regimes (Kavallaris *et al.* J. Clin. Invest. 1997, 100, 1282-1293 and Dumontet *et al.* Bull. Cancer, 2005, 92, E25-30), and a possible β III isotype binding compound has been developed (Ferlini *et al.* 2005, Cancer Res., 65, 2397-2405).

Finally, anti-fungal compound development can be enhanced by utilizing fungal tubulin as a drug target (see also fungal kinesin products on the next page). Currently we offer tubulin from *Agaricus bisporus*, which has good homology to other fungal species. In the future, we will have more fungal tubulin to choose from, please inquire for more details.

Ligand	EC50/IC50 Values			TLI Values	
	Neuronal	HeLa	MCF-7	HeLa	MCF-7
Paclitaxel	0.48	1.04	0.51	0.46	0.94
Docetaxel	0.47	0.41	0.34	1.15	1.38
10-Deacetyl taxol	3.81	30	4.20	0.12	0.88
Vinblastine	1.10	2.83	1.21	0.39	0.91
Vincristine	1.58	2.25	nd	0.70	nd
Colchicine	4.10	3.10	4.60	1.32	0.89
Nocodazole	3.40	3.20	3.20	1.06	1.06
Mebendazole	3.98	25	14.8	0.16	0.27
MF708	3.54	1.91	nd	1.85	nd

IC50 and EC50s were determined based on polymerization reactions containing 2.0 mg/ml tubulin and 0 to 30 μM compound. The CV was 14% for duplicate reactions. To create a dose response graph, the V_{max} of polymerization in the presence of a drug was plotted against Log_{10} of drug concentration. TLI = Tubulin Ligand Index, which is calculated by dividing the EC_{50} from cancer tubulin by the EC_{50} from neuronal tubulin.

Description	Cat. #	Amount
Tubulin Protein, isolated from HeLa cells 90% β I, 10% β IV isotypes	H001-B	1 x 250 μg
Tubulin Protein, isolated from MCF-7 cells 55% β I, 6% β III, 39% β IV isotypes	H005	1 x 250 μg
Tubulin Protein, fungal source <i>Agaricus bisporus</i>	F001	1 x 250 μg

Drug Target: Motor Proteins



Kinesin Drug Discovery

Cytoskeleton, Inc. provides a selection of recombinant kinesin motor-domain proteins from 9 of the 14 reported kinesin classes. The protein domains are useful as targets for anti-mitotic drug discovery and as reagents for comparative studies of kinesin class specific motor activities.

New this year is an Eg5 homolog from the pathogen *Aspergillus fumigatus* (Cat.# EG02), which has potent uses in anti-fungal drug development.

Uses in drug development

- Screening for new anti-tumor ligands
- Screening for new anti-fungal drugs
- Secondary assays for improving specificity
- IC50 measurements for known drugs
- Lead optimization with SAR

Measuring IC50s With the ELIPA™ Realtime ATPase Assay

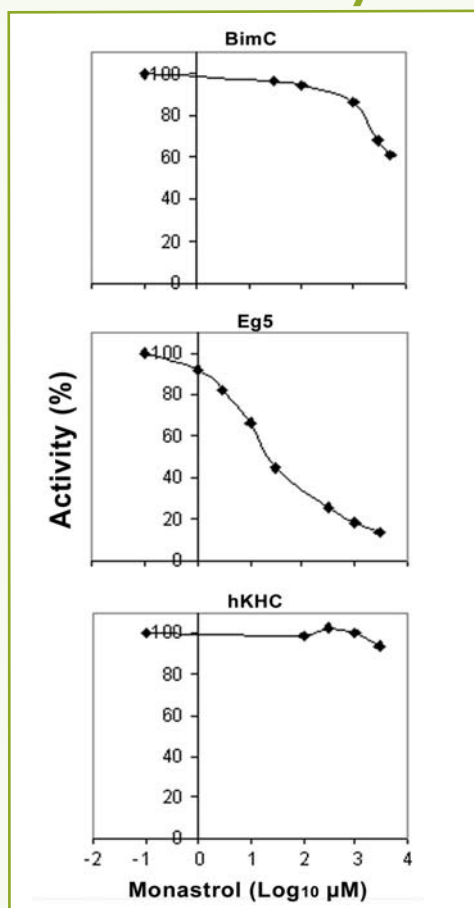


Figure 1. IC50 determination of Monastrol inhibition of kinesin motor activity. Kinesin motors at 1.0, 1.0 and 0.2 μM (BimC, Eg5 and hKHC respectively) were mixed with 4 μM tubulin in the form of microtubules (Cat. # MT001). Reactions were initiated with the addition of ATP at 500 μM . Colorimetric readings were reduced to V_{max} values, which were used to plot the dose-response curves shown above. Further details of the assay process can be obtained from the manual (request at tservice@cytoskeleton.com) or as described in Funk et al. 2004, *Anal. Biochem.*, 329, 68-76.

Pre-formed Microtubules Improve Batch to Batch Reproducibility

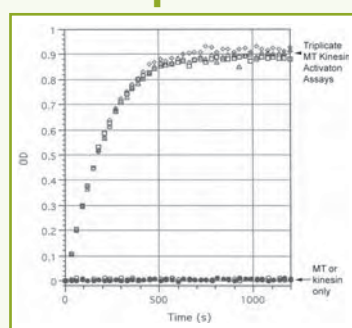


Figure 2. Microtubule activated kinesin ELIPA™ assay using MT001. The CV between samples was <5%.

Detecting Monastrol in a randomized study using the End Point Assay



Purified Motor Proteins

Microtubules

Pre-formed ready to use for kinesin ATPase assays

CENP-E Motor Domain Protein GST-fusion

Source: *H. sapiens*

Chromokinesin Motor Domain Protein GST-fusion

Source: *H. sapiens*

Eg5 Motor Domain Protein GST-fusion

Source: *H. sapiens*

Eg5 Homolog BimC Motor Domain Protein GST-fusion

Source: *A. nidulans*

Eg5 Homolog BimC Motor Domain Protein His-fusion

Source: *A. fumigatus*

NEW Actin Filaments (rabbit skeletal muscle)

Pre-formed ready to use for myosin ATPase assays

KIF3 Motor Domain Protein GST-fusion

Source: *H. sapiens*

KIF3C Motor Domain Protein GST-fusion

Source: *H. sapiens*

Kinesin Heavy Chain Motor Domain Protein GST-fusion

Source: *H. sapiens*

MCAK Motor Domain Protein GST-fusion

Source: *H. sapiens*

MKLP Motor Domain Protein GST-fusion

Source: *H. sapiens*

NEW Myosin Cardiac Muscle Protein

Source: Bovine heart

Myosin II Skeletal Muscle Protein

Source: Rabbit (*O. cuniculus*) skeletal muscle

Heavy Meromyosin Skeletal Muscle Protein

Source: Rabbit (*O. cuniculus*) skeletal muscle

Kinesin ELIPA™ Biochem Kit™

For real time kinetic and V_{max} kinesin ATPase measurements

Kinesin HTS ATPase End Point Assay

For end point measurement of kinesin ATPase activity

Cat

Amount

MT001-A 4 x 500 μg

MT001-XL 1 x 10 mg

CP01-B 10 x 25 μg

CP01-XL 1 x 1 mg

CR01-B 10 x 25 μg

CR01-XL 1 x 1 mg

EG01-B 10 x 25 μg

EG01-XL 1 x 1 mg

BM01-B 10 x 25 μg

BM01-XL 1 x 1 mg

EG02-B 10 x 15 μg

EG02-XL 1 x 1 mg

AKF99-A 1 x 1 mg

AKF99-B 5 x 1 mg

KC01-A 2 x 25 μg

KC01-B 10 x 25 μg

KF01-B 10 x 25 μg

KF01-XL 1 x 1 mg

KR01-B 10 x 25 μg

KR01-XL 1 x 1 mg

MK01-B 10 x 25 μg

MK01-XL 1 x 1 mg

MP01-B 10 x 25 μg

MP01-XL 1 x 1 mg

MY03-A 1 x 1 mg

MY03-B 5 x 1 mg

MY02-A 5 x 1 mg

MY02-B 20 x 1 mg

MH01-A 4 x 50 μg

MH01-B 20 x 50 μg

BK060 96 assays

BK053 1000 assays

Drug Target: Rho Family

Targeting the Rho::ROCK Interaction

Cytoskeleton, Inc. has introduced a range of Rho family protein / effector interaction assays (see also pages 2 and 10). This section describes the protein: protein interaction assays based on purified proteins. This format can identify compounds that disrupt the interaction between a Rho family member and its specific downstream effector. A popular example of this format is the RhoA::ROCK assay. This interaction has been reported to be a possible target for anti-tumor, anti-hypertension drugs and neuroregenerative drugs. It is an upstream target compared to the current target of choice, which is the ROCK kinase domain.

Uses in drug development

- Screening for new anti-hypertensive drugs. (see ref. 1)
- Screening for new anti-tumor drugs (see ref. 2)
- Screening for new neuroregenerative drugs (see ref. 3)
- Secondary assays for improving specificity

Targeting the Rho Family / Effector Interactions

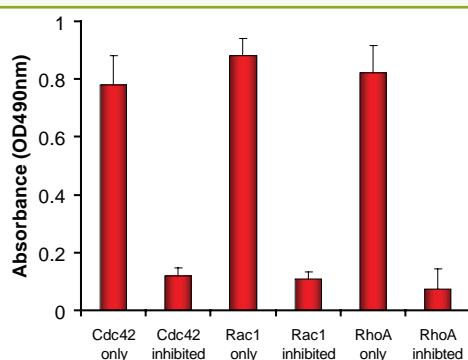


Figure 1. Small G-protein::effector inhibition assay. Effectors for Rho family proteins are covalently bound to wells of a 96-well plate. Compound is added to each well and followed by addition of 10 ng of small G-protein. After washing the wells are developed by a normal ELISA technique with primary and secondary antibodies. Effectors for Cdc42, Rac1 and RhoA are WASP, POSH and ROCK, respectively (Cat. # BK122j, BK122h and BK122d). In this experiment, the inhibitors were 50 nM of PAK, PAK and Rhotekin, respectively.

NOTE: These assays are available on a custom basis only. There is a twelve week production time to provide the completed batch of assays.

Please inquire for a quotation to tservice@cytoskeleton.com or call 303-322-2254 for more information.

Effector Interactions

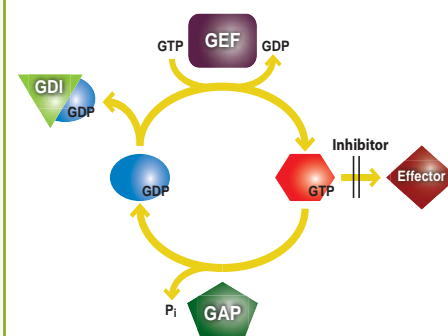


Figure 2. Inhibition of the Rho::effector interaction has great potential to develop a new class of compounds compared to the traditional ROCK kinase inhibitors. Likewise, the Cdc42 and Rac::effector combinations allow other diseases to be targeted with new drug families.

References:

1. Lee et al. 2004, Hypertension, 44, 796-799
2. Gomez del Pulgar et al. 2005, BioEssays, 27, 602-613
3. Mueller et al. 2005, Nat. Rev. Drug Discov., 4, 387-398
4. van Golen et al. 1999, Clin. Cancer Res., 5, 2511-2519.
5. Fryer and Field 2005, Cancer Lett., 229, 13-23.
6. Xu Z. et al. 2003, EMBO J., 22, 252-261.
7. Rao and Li 2004, Curr. Cancer Drug Targets, 4, 345-354.
8. Bollag et al. 2003, Curr. Opin. Investig. Drugs., 4, 1436-1441.
9. Adjei 2001, J. Natl. Cancer Inst. 93, 1062-1074.

Small G-protein HTS assays	Cat. #	Format
G-LISA™ for RhoA::Rhotekin interaction For drug discovery applications see refs. 2 and 3.	BK122a	96 well plate colorimetric
G-LISA™ for RhoB::Rhotekin interaction For drug discovery applications see refs. 2 and 3.	BK122b	96 well plate colorimetric
G-LISA™ for RhoC::Rhotekin interaction Possible use in discovering anti-tumor drugs (ref. 4).	BK122c	96 well plate colorimetric
G-LISA™ for RhoA::ROCK interaction For drug discovery applications see refs 1, 2 and 3.	BK122d	96 well plate colorimetric
G-LISA™ for RhoB::ROCK interaction For drug discovery applications see refs. 2 and 3.	BK122e	96 well plate colorimetric
G-LISA™ for RhoC::ROCK interaction Possible use in discovering anti-tumor drugs (ref. 4).	BK122f	96 well plate colorimetric
G-LISA™ for Rac1::PAK interaction Possible use in discovering anti-angiogenesis drugs (ref. 5)	BK122g	96 well plate colorimetric
G-LISA™ for Rac1::POSH interaction Possible use in discovering anti-apoptotic drugs (ref. 6)	BK122h	96 well plate colorimetric
G-LISA™ for Cdc42::PAK interaction Possible use in discovering anti-angiogenesis drugs (ref. 5)	BK122i	96 well plate colorimetric
G-LISA™ for Cdc42::WASP interaction Possible use in discovering anti-tumor drugs (ref. 7)	BK122j	96 well plate colorimetric
G-LISA™ for H-Ras::Raf Kinase interaction Possible use in discovering anti-tumor drugs (ref. 8, 9).	BK122k	96 well plate colorimetric
G-LISA™ for K-Ras::Raf Kinase interaction Possible use in discovering anti-tumor drugs (ref. 8, 9).	BK122l	96 well plate colorimetric
G-LISA™ for Other Small G-protein::Effector combo	BK122x	96 well plate colorimetric

Detection Method

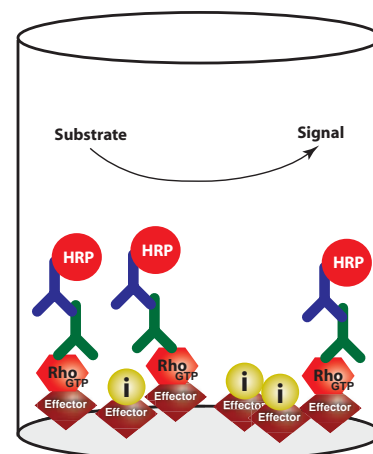


Figure 3. Immobilized effector protein binds the activated form of the small G-protein, which is then detected by standard ELISA technology. Inhibitors (i) reduce the binding of activated small G-protein, which leads to a reduction in signal. Signal to noise ratios are generally 8:1 to 12:1.

Small G-Proteins

New Rac and Rho related products

The mammalian genome contains more than 150 genes for small G-proteins and an even larger number of regulators (such as GEFs GAPs and GDIs) and effectors. These proteins form an intricate network of pathways that orchestrate a multitude of cellular signaling pathways. Understanding these signal transduction networks in both healthy and diseased states is an exciting area of biological research.

Cytoskeleton, Inc. provides a wide range of purified G-proteins, regulators and effectors as well as the most comprehensive array of G-protein signal transduction assay kits available.

New this year are the revolutionary G-LISA™ Rho and Rac activation assays. These are ELISA based activation assays that can be performed in under 3 h. See pages 2, 10 and 17 for more information. Also new this year is the Cell Permeable Rho Inhibitor (Cat. # CT04). C3 transferase (Rho protein

ADP ribosylation factor) has been conjugated to a cell penetrating peptide. This allows rapid and efficient protein delivery into the cell. Once in the cell the peptide is released and C3 transferase is freely available to carry out *in vivo* inhibition of Rho.

C3 cannot normally enter into cells so it has previously been microinjected or incubated overnight (pinocytosis). One is an intricate method the other requires large amounts of C3.

We have shown by cell morphology (F-actin staining with BK005) and Rho Activation Assays (Cat. # BK036 and 124) that in the presence of CT04 cells have reduced Rho activity and their stress fiber morphology changes, see images on p.3. This happens at about 10 fold lower C3 concentrations compared to the un-conjugated protein, and occurs ten times faster (2h compared to 20h). This makes a much more potent and effective Rho inhibitor than siRNA or other current methods (dom. negative protein

injection) because inhibition happens quickly and there are no perturbations of GAP or GEF functions to complicate the results.

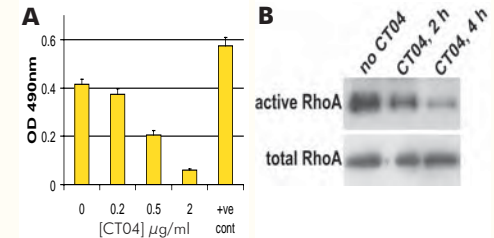


Figure 1. A - Quantitative analysis of Rho inhibition by using the RhoA G-LISA Activation Assay (Cat. # BK124) on cell lysates after CT04 treatment for 4 h at the noted concentrations. B - Traditional Rho Activation Assay using the pull down kit (Cat. # BK036), CT04 was used at 2 μg/ml. Both experiments performed on Swiss 3T3 cells in serum free medium.

Purified, Biologically Active G-Proteins

Cytoskeleton, Inc. produces a wide selection of recombinant wild type and mutant small G-proteins. New this year are Rap1a and Rap1b His tagged wild type proteins (Cat.# RR01 and RR02).

Wild type small GTPases. Biologically active proteins that are tested for their ability to bind and hydrolyze GTP. Uses include:

- Substrate for GEFs, GAPs and effectors.
- Control for activated and dominant negative mutants

Activated mutants of small G-proteins: Constitutively activated mutants of small G-protein have mutations that render them GTPase inactive. Because of this they are always activated. These mutants are useful tools for:

- Affinity reagents for effector proteins and GAPs.
- Microinjections to examine the phenotype of the activation of the G-protein in cells.

Dominant negative mutants of small G-proteins: Dominant negative mutants of small G-proteins bind firmly to GEFs and block the GEFs from binding to and activating the endogenous G-proteins. They can be used for:

- Affinity reagent for GEFs.
- Microinjections to block the activity of the endogenous G-protein in cells.



Figure 2. Purified His-tagged Cdc42 protein (Cat. # CD01). The protein was run on an SDS-PAGE gel and coomassie stained. This protein was also shown to be active both in nucleotide exchange assays and in GTPase assays.

Purified G-Proteins	Cat. #	Amount
Cdc42 His Protein, constitutively active (Q61L)	C6101-A	1 x 10 μg
	C6101-C	4 x 10 μg
Cdc42 GST Protein, dominant negative (T17N)	C17G01-A	1 x 25 μg
	C17G01-C	4 x 25 μg
Cdc42 GST Protein, wild type	CDG01-C	8 x 25 μg
Cdc42 His Protein, wild type	CD01-A	1 x 100 μg
	CD01-C	3 x 100 μg
Rac1 His Protein, constitutively active (Q61L)	R6101-A	1 x 10 μg
	R6101-C	4 x 10 μg
Rac1 GST Protein, dominant negative (T17N)	R17G01-A	1 x 25 μg
	R17G01-C	4 x 25 μg
Rac1 GST Protein, wild type	RCG01-C	8 x 25 μg
Rac1 His Protein, wild type	RC01-A	1 x 100 μg
	RC01-C	3 x 100 μg
Ran His Protein, constitutively active (Q69L)	RN03-A	1 x 10 μg
	RN03-B	3 x 10 μg
Ran His Protein, dominant negative (T24N)	RN05-A	1 x 10 μg
	RN05-B	3 x 10 μg
Ran His Protein, wild type	RN01-A	1 x 10 μg
	RN01-B	3 x 10 μg
Rap1a His Protein, wild type	RR01-A	1 x 100 μg
	RR01-B	3 x 100 μg
Rap1b His Protein, wild type	RR02-A	1 x 100 μg
	RR02-B	3 x 100 μg
Ras: H-Ras GST Protein, constitutively active (G12V)	GV12G01-A	2 x 50 μg
	GV12G01-C	8 x 50 μg
Ras: H-Ras His Protein, wild type	RS01-A	1 x 100 μg
	RS01-C	3 x 100 μg
RhoA His Protein, constitutively active (Q63L)	R6301-A	1 x 10 μg
	R6301-C	4 x 10 μg
RhoA GST Protein, wild type	RHG01-C	8 x 25 μg
RhoA His Protein, wild type	RH01-A	1 x 100 μg
	RH01-C	3 x 100 μg
RhoC His Protein, wild type	RH03-A	1 x 100 μg
	RH03-C	3 x 100 μg

and Signal Transduction

Small G-protein Antibodies

New this year is a Rac1 specific antibody that reacts with Rac1 in all metazoan tissue samples currently tested, but does not bind to Rac2, Rac3 or Cdc42 as other commercially available "Rac1" antibodies do.

QC Max™ antibodies have been thoroughly tested in Western blots, immunoprecipitation, ELISA, immunohistochemistry and species cross reactivity. Because of this, Cytoskeleton's small G-protein antibodies are some of the most well-characterized antibodies available.

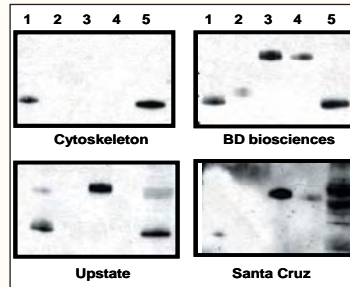


Figure 3. Specificity and sensitivity of ARC03. ARC03 does not cross-react with Rac2, 3 or Cdc42 (upper left blot), while all other commercially available Rac1 antibodies crossreact with GTPases other than Rac1. The blots were probed with a 1 µg/ml (1:500) dilution of antibody. Ln1, His-Rac1; Ln2, His-Rac2; Ln3, Rac3-GST; Ln4, Cdc42-GST and Ln5, 50 µg of platelet extract.

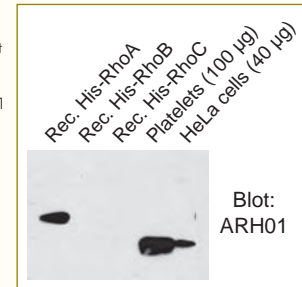
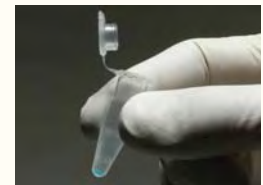


Figure 4. Specificity and sensitivity of ARH01. Recombinant RhoA, B, and C as well as platelet and HeLa lysates were run on an SDS-PAGE gel and blotted with ARH01. Zooblots indicate detection of RhoA in species as diverse as nematode to human.

G-Protein Modulator and Effector Proteins	Cat. #	Amount
NEW Cell Permeable Rho Inhibitor Just add to tissue culture media to inhibit Rho in 2 to 4h.	CT04-A CT04-B CT04-C	1 x 20 µg 5 x 20 µg 20 x 20 µg
C3 Transferase Protein Specific inhibitor of Rho activity	CT03-A CT03-C	1 x 25 µg 4 x 25 µg
Dbs His Protein, RhoGEF domain (DH/PH) GEF for Cdc42 and RhoA	GE01-A GE01-B	2 x 50 µg 8 x 50 µg
p50RhoGAP GST Protein, full length GAP for Cdc42, Rac and Rho	GAP01-A GAP01-B	1 x 50 µg 4 x 50 µg
p50RhoGAP GST Protein, GAP domain GAP for Cdc42, Rac and Rho	GAS01-A GAS01-B	1 x 50 µg 4 x 50 µg
PAK-PBD Protein Binds specifically to active (GTP-bound) Cdc42 and Rac	PAK01-A PAK01-B	1 x 250 µg 4 x 250 µg
PAK-PBD Beads Binds specifically to active (GTP-bound) Cdc42 and Rac	PAK02-A PAK02-B	1 x 500 µg 4 x 500 µg
Raf-RBD Beads Binds specifically to active (GTP-bound) Ras	RF02-A RF02-B	1 x 2 mg 4 x 2 mg
RanBP1 His Protein Binds specifically to active (GTP-bound) Ran	RN07-A RN07-B	1 x 250 µg 3 x 250 µg
RhoGDI GST Protein Inhibitor of Cdc42, Rac and Rho	GDI01-A GDI01-B	1 x 25 µg 4 x 25 µg
Rhotekin-RBD Protein Binds specifically to active (GTP-bound) Rho	RT01-A RT01-B	1 x 500 µg 3 x 500 µg
Rhotekin-RBD Beads Binds specifically to active (GTP-bound) Rho	RT02-A RT02-B	2 x 2 mg 6 x 2 mg
WASP-CBD Beads Binds specifically to active (GTP-bound) Cdc42	WS03-A WS03-B	1 x 250 µg 3 x 250 µg

Rhotekin-RBD and PAK-PBD

Easy to see format (colored beads)



Cytoskeleton provides Rhotekin-RBD, PAK-PBD and a variety of other effector domains that specifically target the active form of different small G-proteins (see table to the left). These domains can be used for:

- RhoA, RhoB or RhoC Activation assays (Cat.# RT02)
- Rac1, Rac2 or Rac3 Activation assays (Cat.#PAK02)
- Cdc42 Activation assays (Cat.# PAK02 or WS03)

Antibodies to Small G-proteins	Cat. #	Amount
NEW Rac1 Specific Antibody Mouse monoclonal, affinity purified	ARC03-A ARC03-B	2 x 50 µg 6 x 50 µg
Rac Antibody Rabbit polyclonal, affinity purified	ARC01-A ARC01-B	1 x 50 µg 3 x 50 µg
RhoA Antibody Mouse monoclonal, affinity purified	ARH01-A ARH01-B	1 x 50 µg 3 x 50 µg

Other Signal Transduction Reagents	Cat. #	Amount
EasyRad™ GTPase Assay Simple one step radioactive assay (Kcat >0.0001)	BK055	1000 assays
GTPγS Non-hydrolyzable GTP analog, 50 µl of 20 mM	BS01	1 x 500 µg
GTPase ELIPA™ Assay Enzyme linked inorganic phosphate assay provides a non-radioactive method for quantitating GTPase activity	BK052	96 assays
Phalloidin (rhodamine labeled 14 µM) Stabilizes and selectively labels actin filaments	PHDR1	1 x 500 µl

G-protein Biochem Kits™

Cytoskeleton, Inc. provides a wide range of Biochem Kits™ for the assay of small G-proteins and signal transduction.

These include our new G-LISA™ Rho and Rac activation assays, GEF and GAP assay kits as well as our well documented pulldown activation assays.

See pages 2, 10-11, 17 and 18 for more information on these products

RhoGEFs, RhoGAPs and RhoGDIs

Cytoskeleton offers a variety of these proteins (see table to the left) and they can be used for:

- *In vitro* screens for inhibitors of GEFs and GAPs
- Positive controls in *in vitro* GEF and GAP assays testing for specificity of novel GEFs and GAPs
- Inhibition and activation of small G-proteins in cells and *in vitro*

Please inquire for bulk discounts for drug discovery projects

Tubulin Proteins

About Tubulin Products

Tubulin is a major structural protein that is highly conserved between species and is present in virtually all eukaryotic cells. The basic tubulin subunit consists of a heterodimer of one α - and one β - isotype. Heterodimers can polymerize *in vivo* and *in vitro* to form microtubules (MTs). *In vivo*, MTs are regulated by a large number of microtubule binding proteins

(MAPs) that are temporally and spatially coordinated to orchestrate the activity of a highly dynamic MT cytoskeleton. The MT cytoskeleton functions in a large number of cellular processes, including cell shape, motility, intracellular transport and cell division. Tubulin is also an important therapeutic target for antimetabolic drugs in the treatment of cancers.

New in 2007 is a fluorescent conjugate, X-rhodamine tubulin (Cat.# TL334M). This tubulin conjugate is specifically tailored to live cell imaging and dual stain live cell imaging where the far red wavelength allows for greater separation between fluorescein or Alexa 488 and X-rhodamine.

Pure Tubulin

The highly conserved structure of tubulin allows the use of porcine or bovine brain protein as the standard *in vitro* reagent for both basic research and drug discovery applications. Cytoskeleton is the world leader in providing purified tubulins (>90% to >99% pure) for your research needs. Inquire to technical assistance for a product citation in your area of interest. Tubulins are provided from:

- Bovine brain (neuronal) tubulin.
- Porcine brain (neuronal) tubulin.
- Cancer cell (ovarian, breast and cervical) tubulin.
- Fungal cell (*A. bisporus*) tubulin.

Uses for purified tubulin proteins

- Tubulin monomer binding studies, using Cat. # TL238 or T240. Hung *et al.* 2004, Mol. Biol. Cell, 15, 2697-2706.
- Microtubule binding studies, using TL238, T240 or T238. Farah *et al.* 2005, J. Biol. Chem., 280, 9439-9449.
- HDAC6 studies, using Cat. # TL238 or T240. Haggarty *et al.* 2003, PNAS, 100, 4389-4394.
- Drug discovery studies using Cat. # HTS02, ML113 or ML116. Kasibhatla *et al.* 2004, Mol. Cancer Ther. 3, 1365-74.
- IC50 determinations using Cat. #s TL238, T240. Mooberry *et al.* 1999, Cancer Res., 59, 653-660.
- Kinesin ATPase assays using Cat. # MT001. Corporate references on request.
- Disease targeted tubulin ligand development using Cat. # F001, H001, H005, H011 or H015. Giannakakou *et al.* 1997, J. Biol. Chem., 272, 17118-17125.

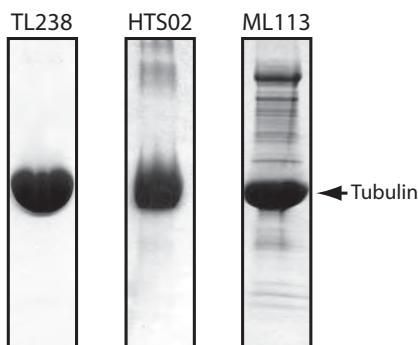


Figure 1. Tubulin purity. >99% pure tubulin (TL238), HTS-tubulin (HTS02), and MAP rich tubulin (ML113) were run on SDS-PAGE gels and stained with coomassie blue.

Specialized Tubulins

There are instances where specialized tubulins from a non-neuronal or a non-mammalian source are valuable. For example, this can be useful in searching for drugs that are targeted towards a dividing cancer cell or fungal infections. For this purpose, Cytoskeleton has developed specialized tubulins that can be used to screen for tubulin drugs that target a certain type of microtubule network.

Our specialized tubulin products include:

- Cancer cell tubulins (H001-B & H005). Nunes *et al.* 2005, Biochemistry, 44, 6844-6857
- Biotinylated cancer cell tubulins (H003 & H005)
- Fungal tubulin (F001) Am. Soc. Cell Biol. Ann. Mtg. 2005 Program Abstract # 964
- FtsZ, a bacterial tubulin homolog (FTZ01) Lu *et al.* 2001, BMC Microbiology, 1, 7

Tubulin Proteins

Tubulin Protein

Porcine brain, >99% pure, lyophilized

Cat.

Amount

T240-A 1 x 1 mg
T240-B 5 x 1 mg
T240-C 20 x 1 mg
T240-DX 1 x 10 mg
T240-XL Large quantities

Tubulin Protein

Bovine brain, >99% pure, lyophilized

TL238-A 4 x 250 μ g
TL238-B 1 x 1 mg
TL238-C 5 x 1 mg
TL238-D 10 x 1 mg
TL238-DX 1 x 10 mg
TL238-E 20 x 1 mg
TL238-F Large quantities

Tubulin for HTS Applications

Bovine brain, >97% pure, lyophilized

HTS02-A 1 x 4 mg
HTS02-B 1 x 40 mg
HTS02-XL 1 x 100 mg

Microtubules

pre-formed and ready to use, lyophilized

MT001-A 4 x 500 μ g
MT001-XL 1 x 10 mg

Tubulin Protein: MAP rich

Bovine brain, 70% tubulin, 30% MAPs, lyophilized

ML113-B 1 x 1 mg
ML113-C 5 x 1 mg
ML113-D 10 x 1 mg
ML113-DX 1 x 10 mg
ML113-E 20 x 1 mg
ML113-XL Large quantities

Tubulin Protein, MAP rich

Porcine brain, 70% tubulin, 30% MAPs, lyophilized

ML116-A 1 x 1 mg
ML116-B 5 x 1 mg
ML116-C 20 x 1 mg
ML116-DX 1 x 10 mg
ML116-XL Large quantities

Tubulin Protein

Cancer cell tubulin, HeLa cells

H001-B 1 x 250 μ g

Tubulin Protein

Cancer cell tubulin, MCF-7 cells

H005 1 x 250 μ g

Tubulin Protein

Fungal tubulin, *Agaricus bisporus*

F001 1 x 250 μ g

FtsZ Protein

Bacterial tubulin homolog

FTZ01-A 1 x 1 mg
FTZ01-B 5 x 1 mg

Tubulin Protein

Bovine brain, >99% pure, frozen liquid

T238-A 4 x 250 μ g
T238-B 1 x 1 mg
T238-C 5 x 1 mg
T238-E 20 x 1 mg

Tubulin plus glycerol

Bovine brain, >99% pure, frozen liquid

T237-A 4 x 250 μ g
T237-B 1 x 1 mg
T237-C 5 x 1 mg
T237-E 20 x 1 mg

Labeled Tubulin Proteins

Labeled Tubulins

Cytoskeleton provides highly pure (>99%) and biologically active biotin, fluorescein and rhodamine labeled tubulins from different sources. Uses for these include:

- Monitoring microtubule dynamics in living cells by microinjection (fluorescein and rhodamine tubulin). Schaefer *et al.* 2002, *J. Cell Biol.*, 158, 139-152.
- Studying motors and MAPs activity *in vitro* with fluorescent microtubules. Cho *et al.* 2005, *Mol. Cell Biol.*, 25, 4541-4551.
- Nanotechnology Hirst *et al.* 2005, *Langmuir*, 21, 3910-3914.
- Tubulin binding assays using SPA beads or streptavidin coated plates (biotin tubulin, see page 15)



Figure 2. Rhodamine microtubules.

Tubulin Biochem Kits™ and HTS assays

Cytoskeleton, Inc. is the world leader in providing an extensive range of highly robust Tubulin Biochem Kits™ and high throughput format CytoDYNAMIX™ screens. Biochem Kits™ and CytoDYNAMIX™ screens provide all the proteins, antibodies, and buffers required to perform the chosen assay. Each assay technique is backed up by full technical service, which you can use to optimize your assay under the conditions you require, or modify the assay for the apparatus that you have available. See pages 8 (Biochem Kits™) and 14-15 (CytoDYNAMIX™ screens) for more information.

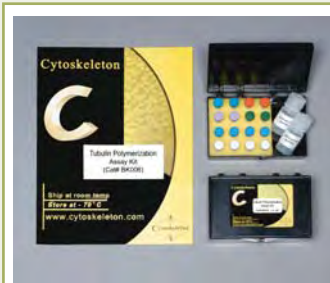


Figure 3. Tubulin Polymerization Biochem Kit™ (Cat. # BK006).

Pre-formed Microtubules

Microtubule (MT) substrates are required in the study of a wide range of Microtubule Associated Proteins (MAPs). The formation of MTs *in vitro* is a complex process in which the length and final polymer mass of MTs is greatly affected by experimental conditions, including temperature, tubulin purity, tubulin concentration, timing of the polymerization reaction, timing of paclitaxel addition and buffer conditions.

Scientists at Cytoskeleton, Inc. have developed a patented method for MT production and lyophilization that ensures highly reproducible MTs (2 μm average length). Pre-formed microtubules (Cat. # MT001) provide an extremely convenient way to perform assays requiring microtubules as a substrate. Microtubules are supplied as a stable lyophilized powder, simply resuspend and use. Uses for pre-formed stabilized microtubules include:

- Substrate for discovery and characterization of microtubule binding proteins (MAPs)
- Substrate for activation of kinesin ATPase
- Determination of IC50s for kinesin inhibitors
- Used in Cytoskeleton, Inc.'s kinesin ELIPA and end point ATPase assays (BK060 & BK053)
- Ideal for HTS applications (inquire for bulk rates). Corporate references on request.

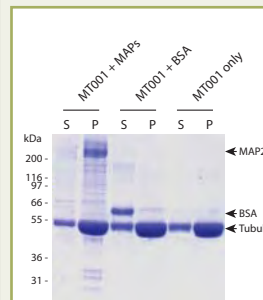


Figure 4. MT binding spin-down assay using MT001. >80% of MT001 (arrow: Tubulin) is in pellet (P) after spin-down. MAPs bind to MTs and end up in pellet while BSA does not and stays in supernatant (S).

Labeled Tubulin Proteins

	Cat. #	Amount
Biotin Tubulin Bovine brain, lyophilized	T333-A T333-B T333-XL	5 x 20 μg 20 x 20 μg 1 x 500 μg
Biotin Tubulin Cancer cell (HeLa), lyophilized	H003	1 x 40 μg
NEW Biotin Tubulin Cancer cell (MCF-7), lyophilized	H007	1 x 40 μg
Fluorescein Tubulin minus glycerol (Ex485, Em535) Bovine brain, frozen liquid	T332M-A T332M-B	5 x 20 μg 20 x 20 μg
Rhodamine Tubulin (Ex535, Em585) , bovine brain, lyophilized. Recommended for microinjections and general use.	TL331M-A TL331M-B	5 x 20 μg 20 x 20 μg
Rhodamine Tubulin minus glycerol (Ex535, Em585) . Bovine brain, frozen liquid.	T331M-A T331M-B	5 x 20 μg 20 x 20 μg
Rhodamine Tubulin plus glycerol (Ex535, Em585) . Bovine brain, frozen liquid.	T331-A T331-B	5 x 20 μg 20 x 20 μg
NEW X-rhodamine Tubulin (Ex570, Em610) . Bovine brain, lyophilized. Recommended for microinjections and general use.	TL334M-A TL334M-B	5 x 20 μg 20 x 20 μg

Tubulin Buffers, Reagents & MAPs

	Cat. #	Amount
General Tubulin Buffer Lyophilized	BST01-001 BST01-010 BST01-100	1 x 10 ml 1 x 100 ml 1 x 1000 ml
GTP (100 mM stock) Lyophilized	BST06-001 BST06-010	1 x 100 μl 10 x 100 μl
Tubulin Glycerol Buffer Enhances tubulin polymerization	BST05-001	1 x 10 ml
Microtubule Associated Protein (MAP) Fraction Bovine brain MAP fraction, 70% MAP2	MAPF-A MAPF-C	1 x 100 μg 5 x 100 μg
Paclitaxel (2 mM stock) Stabilizes microtubules.	TXD01	10 x 100 μl
Tau Protein Bovine brain, lyophilized	TA01-A TA01-B	1 x 50 μg 3 x 50 μg

Tubulin Antibodies

	Cat. #	Amount
Tubulin Antibody, α & β isotype Affinity purified sheep polyclonal. Reacts with all species, yeast to human	ATN02-A ATN02-B	1 x 100 μg 3 x 100 μg
Anti-sheep IgG HRP conjugate	SG01	1 x 500 μg
Anti-sheep IgG Rhodamine conjugate	SG02	1 x 500 μg

Tubulin Antibodies

Cytoskeleton, Inc. has developed a pan-specific tubulin antibody (ATN02) based on the requirement for probing cell lines and tissues for multiple antigens. ATN02 is a sheep polyclonal antibody, hence it can be co-incubated with mouse, rat or rabbit antibodies for selective microtubule staining.

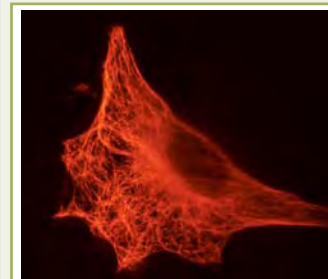


Figure 5. Staining of the tubulin network of a cell with Cytoskeleton's sheep anti-tubulin antibody (ATN02).

Advanced Protein Assay™



Uses include measuring protein in:

- **SDS-PAGE Loading Buffer**
- **Low Protein Concentration Solutions**
- **Column Fractions**
- **Purified Proteins and Antibodies**

Detergent Compatible*

- **Triton X-100**
- **NP40 or Igepal**
- **Tween 20**
- **SDS**

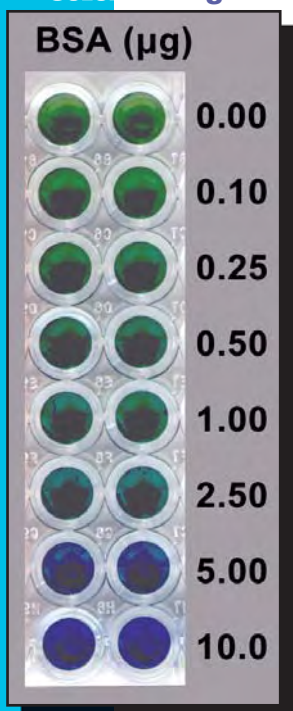
* = Based on 2 μ l sample in 300 μ l of reagent.

Over the past 10 years Cytoskeleton Inc. has developed two protein assays with a wide range of applications. The Advanced Protein Assay Reagent (Cat. # ADV01) is useful for measuring small quantities of protein in the 0.25 to 10 μ g range.

The assay is useful for low level expressed proteins

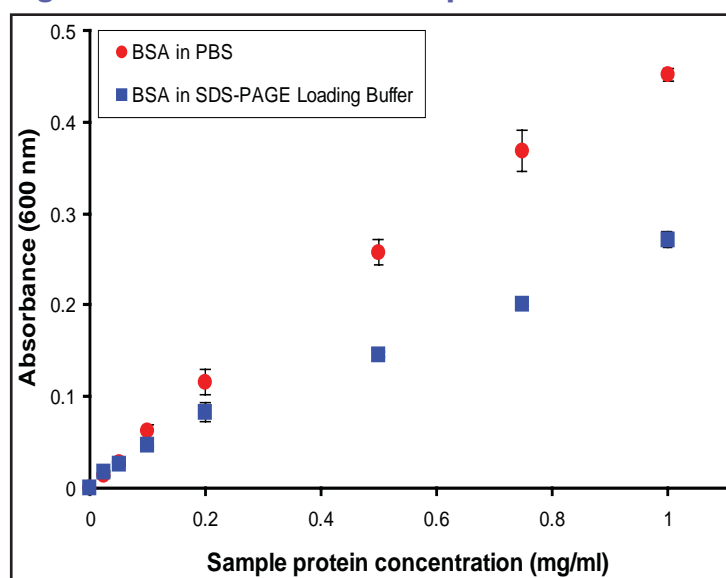
as well as small amounts of total protein from a cell lysate. A further application of this reagent is to measure protein that is already dissolved in SDS-PAGE loading buffer. This application is especially useful for samples that are automatically prepared in 96-well format and need quantifying prior to gel analysis.

Color change



Measuring Protein in SDS-PAGE Sample Buffer

Figure 1. Comparison of protein measurements in PBS buffer and SDS-PAGE sample buffer. 2 μ l of sample plus 300 μ l of reagent were mixed in a well of a standard 96-well plate. Absorbance at 600 nm was read and plotted on the figure. ADV01 gives a linear response in PBS and SDS-PAGE sample buffer. Note: The coefficient does change and measurements in SDS-PAGE sample buffer should be compared to standards in the same buffer.



Description	Cat. #	Amount
Advanced Protein Assay (5 x stock reagent)	ADV01-A	1 x 500 ml
Colorimetric protein assay. Quantitates protein solutions in the range of 25-1000 μ g/ml	ADV01-B	3 x 500 ml

Precision Red Protein Assay™

Uses include, measuring protein in:

- Cell Extracts
- Serum Samples
- High Protein Concentration Solutions
- Proteins Requiring GLP or Higher Standards
- Purified Proteins and Antibodies

Detergent compatible

- Triton X-100
- NP40 or Igepal
- Tween 20
- SDS (limited)



The Precision Red Protein Assay Reagent was developed to achieve the same low protein to protein variation and detergent compatibility seen with the Advanced Protein Assay, plus a longer linear range to allow a wide variety of samples to be read with ease. A simple one step reagent addition combined with a short color devel-

opment time (1 min) allows the user to analyze many samples with confidence.

This reagent is so reproducible at a wide range of protein concentrations that it is employed to accurately determine the concentration in all of Cytoskeleton, Inc's protein products.

Linearity of Precision Red Protein Measurements

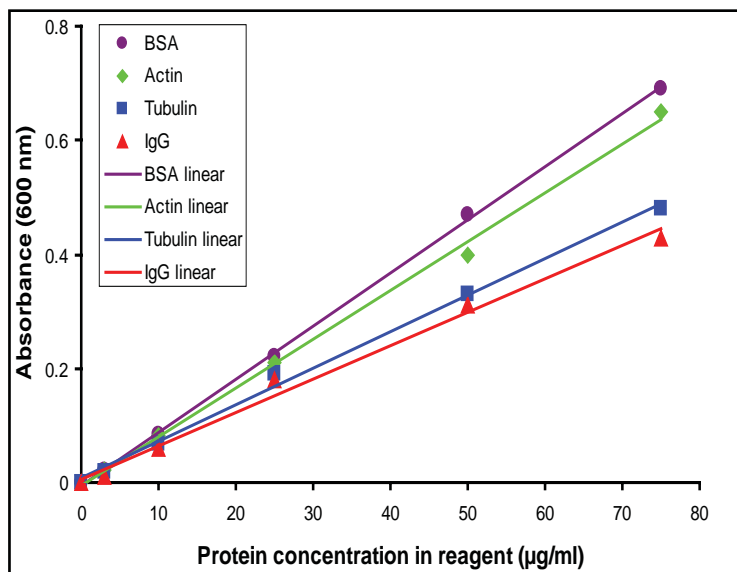
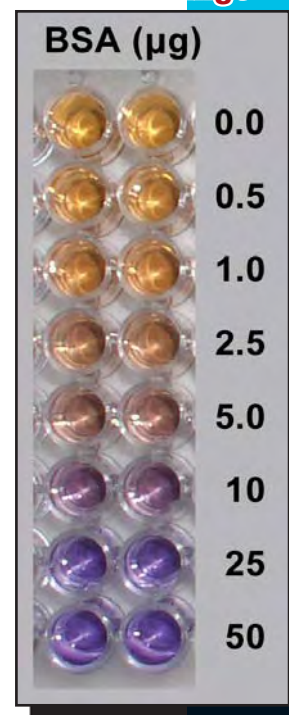


Figure 2. Comparison of different proteins measured over a wide range of concentrations. 10 µl of sample plus 1 ml of reagent were pipetted into a 1.0 ml cuvette and incubated for 1 min. Samples were read in a spectrophotometer set at 600 nm and the data plotted on the figure. ADV02 gives a linear response from 0.25 mg/ml to 50 mg/ml in PBS or Tris buffer. Samples can also be measured in 96-well format using 5 or 10 µl of sample and 300 µl of reagent.

Color change



Description	Cat. #	Amount
Precision Red™ Advanced Protein Assay (1 x stock reagent)	ADV02-A	1 x 500 ml
Colorimetric protein assay. Quantitates protein solutions in the range of 0.25-50 mg/ml	ADV02-B	3 x 500 ml



Revolutionary New Products

Rac and Rho Activation Assays

The days of laborious Small G-protein activation assays are over. Using the G-LISA format you can assay up to 96 samples in one day!

1. Rapid (less than 3 hours assay time)
2. Quantitative (numerical output easily compared)
3. Sensitive (10 to 50 μg of total cell protein)
4. Flexible detection modes (luminescence or colorimetric)

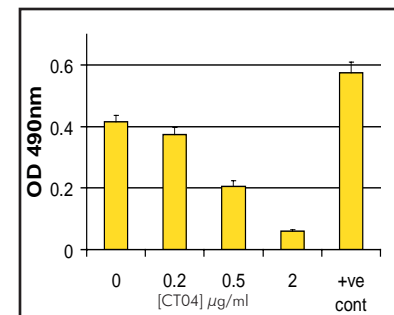


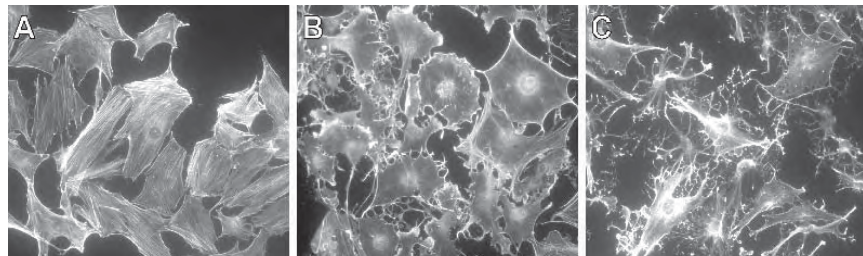
Figure shows signals obtained from HeLa cells treated with different amounts of Rho inhibitor, Cat.# CT04. Activation Assays Cat.# BK121, BK123, BK124, BK125, BK126 (see pages 2, 10 and 18).

Cell Permeable / Rapid Rho Inhibitor

Don't waste time with slow and misleading siRNA inhibition of Rho. Use the potent, specific and rapid cell permeable Rho inhibitor from Cytoskeleton, Inc.

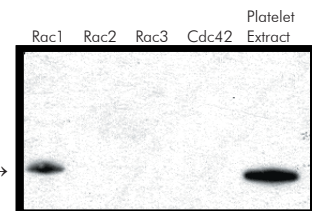
1. Specific (C3 transferase based)
2. Potent (Inhibit upto 80% of cellular Rho)
3. Rapid (effective within 2 hours)

The effects are also titratable to suit your application (see the figure opposite). Panel A - Control Swiss 3T3 cells, Panel B - Use low concentrations for morphological effects, Panel C - Use high concentrations for strong inhibition and biochemical assays. Cat. # CT04 (see pages 3 and 18).



Anti-Rac1 Specific Antibody

Other commercially available Rac antibodies do not distinguish between Rac1 and Rac2, Rac3 or Cdc42. Cytoskeleton Inc. has developed a Rac1 specific monoclonal antibody (Cat. # ARC03) that has >100 fold specificity compared to Rac3 and >1000 fold specificity compared to Rac2, Cdc42 and H-Ras (see pages 3,19).



Distributor information