

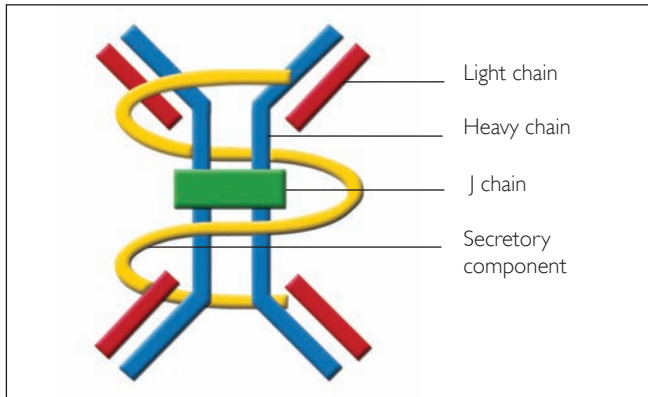


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IMMUNOGLOBULIN A

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The mucosal surfaces represent the largest area of exposure of the body to external pathogens. Immunoglobulin A (IgA), in its secretory form, is the main effector of the mucosal immune system and provides an important first line of defense against most pathogens that invade the body at a mucosal surface¹. Secretory IgA (SIgA) represents the most abundant immunoglobulin of body secretions such as saliva, tears, colostrum and gastrointestinal secretions. The molecular stability and effector immune functions make SIgA particularly well suited to provide mucosal protection against pathogens.



SIgA is produced by plasma cells predominantly as polymeric IgA (pIgA) consisting of two or more monomers linked by the J (joining) chain. pIgA is actively transported by the epithelial polymeric Ig receptor (pIgR) and released into mucosal secretions with a bound secretory component (the extracellular domain of the pIgR) that protects the molecule from proteolytic enzymes. IgA mediates a variety of protective functions²⁻³. Luminal SIgA is believed to interfere with pathogen adherence to mucosal epithelial cells, a process called immune exclusion. In addition, IgA appears to have two other defense functions: intracellular neutralization, and virus excretion. IgA is also found as a monomer in the serum where it may function as a second line of defence by eliminating pathogens that have breached the mucosal surface. Serum IgA interacts with an Fc receptor called Fc α R1 triggering antibody-dependent-cell-mediated cytotoxicity (ADCC).

Due to their specific effector functions, IgA present an interesting therapeutic potential for mucosal protection against virus and bacteria. Indeed, monoclonal IgA antibodies have been shown to be efficient in protecting against infection by various bacteria and viruses, including HIV-1⁴⁻⁶.

Despite this great potential and in contrast to monoclonal antibodies (MAbs) of the IgG isotype, their development as research tools or human therapeutics has been scarce. This is mostly due to the difficulties encountered in producing and purifying biologically active IgA. IgA MAbs can hardly be obtained through the classical hybridoma technique that involves the fusion between murine splenocytes and myeloma cells⁷. Studies of IgA would be much facilitated by the availability of a simple method to isolate and detect IgA.

As a specialist of the Toll-like receptors (TLRs) and innate immunity, InvivoGen believes that IgA is a new hot topic in this field and therefore is initiating a vast IgA program. In this regard, we are using two innovative methods to generate IgA MAbs. The first relies on the use of a transgenic mouse, named C α , obtained through insertion of the human α I gene in place of the switch sequence S μ 7, that allows the isolation of primarily chimeric IgA MAbs via the classical hybridoma technique. The second combines hybridoma and recombinant DNA technologies and involves an IgG-IgA class-switch. In both methods, mice are DNA immunized with a plasmid expressing the antigen, and IgA- or IgG-producing hybridomas are screened using a neutralizing assay based on engineered cell lines (HEK-Blue™ Cells). IgA antibodies are purified by Protein L affinity chromatography. Protein L is a bacterial protein that binds antibodies through κ light chain interactions. These techniques have been utilized to generate a first series of IgA MAbs that target the extra-cellular TLRs and key cytokines of the innate immune system. These IgA MAbs display potent neutralizing activities and can be used for flow cytometry, and thus represent useful research tools. Many more IgA MAbs are in the pipeline, some with potential therapeutic applications.

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3. Snoeck V. et al., 2006. The IgA system: a comparison of structure and function in different species. *Vet Res.* 37(3):455-67. Review.
4. Yan H. et al., 2002. Multiple functions of immunoglobulin A in mucosal defense against viruses: an in vitro measles virus model. *J. Virol.* 76:10972.
5. Huang YT. et al., 2005. Intraepithelial Cell Neutralization of HIV-1 Replication by IgA. *J. Immunol.* 174: 4828 - 4835.
6. Mantis NJ. et al., 2007. Inhibition of HIV-1 Infectivity and Epithelial Cell Transfer by Human Monoclonal IgG and IgA Antibodies Carrying the b12 V Region. *J. Immunol.* 179: 3144 - 3152.
7. Cogne M. et al., 2007. Non-Human Transgenic Mammal for the Constant Region of the Class a Human Immunoglobulin Heavy Chain and Applications Thereof. US2007248601.

IgA Product Line

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IgA Antibodies

Description

Our monoclonal IgA antibodies are chimeric antibodies in which the constant domains of the human IgA molecule were combined with murine variable regions. They have been selected for their ability to efficiently neutralize the biological activity of selected cytokines and Toll-Like Receptors. The neutralizing activity of these IgA antibodies was determined using InvivoGen's HEK-Blue™ Cytokine Cells or HEK-Blue™ TLR Cells. Most of InvivoGen's monoclonal IgA antibodies can also be used for flow cytometry.

Contents and Storage

Each neutralizing IgA antibody is provided lyophilized from a 0.2 µm filtered solution in PBS. Product should be reconstituted in sterile water. Lyophilized antibodies are stable greater than six months when stored at -20°C. Reconstituted IgAs are stable 1 month when stored at 4°C and 6 months when aliquoted and stored at -20°C.

Anti-Cytokine IgAs

ANTIBODY	REACTIVITY	APPLICATIONS	QUANTITY	CATALOG CODE
Anti-hCD40L-IgA NEW	Human CD40L	Neutralization	100 µg	maba-hcd40l
Anti-hIFNα-IgA	Human IFN-α	Neutralization, FC	100 µg	maba-hifna
Anti-hIFNγ-IgA NEW	Human IFN-γ	Neutralization	100 µg	maba-hifng
Anti-hIL-1β-IgA	Human IL-1β	Neutralization	100 µg	maba-hil1b
Anti-hIL-4-IgA	Human IL-4	Neutralization	100 µg	maba-hil4
Anti-hIL-6-IgA	Human IL-6	Neutralization, FC	100 µg	maba-hil6
Anti-hIL-10-IgA NEW	Human IL-10	Neutralization	100 µg	maba-hil10
Anti-hIL-13-IgA	Human IL-13	Neutralization, FC	100 µg	maba-hil13
Anti-hIL-18-IgA NEW	Human IL-18	Neutralization	100 µg	maba-hil18
Anti-hTGFβ-IgA NEW	Human TGF-β	Neutralization	100 µg	maba-htgfb
Anti-hTNFα-IgA	Human TNF-α	Neutralization, FC	100 µg	maba-htnfa

Anti-TLR IgAs

ANTIBODY	REACTIVITY	APPLICATIONS	QUANTITY	CATALOG CODE
Anti-hCD14-IgA	Human CD14	Neutralization of TLR2 and TLR4, FC	100 µg	maba-hcd14
Anti-hTLR2-IgA	Human TLR2	Neutralization, FC	100 µg	maba2-htlr2
Anti-hTLR3-IgA	Human TLR3	FC	100 µg	maba-htlr3
Anti-hTLR4-IgA	Human TLR4	Neutralization, FC	100 µg	maba-htlr4
Anti-hTLR5-IgA	Human TLR5	Neutralization, FC	100 µg	maba2-htlr5

Control IgA

ANTIBODY	REACTIVITY	APPLICATIONS	QUANTITY	CATALOG CODE
IgA2 Isotype Control	Human IgA2	Control	100 µg	maba2-ctrl

Secondary Antibodies

InvivoGen provides F(ab')₂ fragment secondary antibodies, to avoid non-specific binding through Fc receptors, that react with human IgA. These goat antibodies are conjugated with fluorescein (FITC) or biotin for immunodetection or cell sorting applications. Secondary anti-human IgA antibodies are supplied in 1 ml PBS/NaN₃. Store at 4°C.

PRODUCT	QTY	CAT. CODE
Goat F(ab')₂ Anti-Human IgA - Biotin	500 µg	chiga-biot
Goat F(ab')₂ Anti-Human IgA - FITC	500 µg	chiga-fitc
Goat F(ab')₂ IgG Isotype Control - FITC	100 tests	cgig-fitc

6 IgA Antibody Purification

Protein G and Protein A are currently used to purify IgG antibodies, but these supports are not appropriate for IgA antibody purification. For fast and efficient purification of IgA antibodies from biological samples, InvivoGen provides now four different methods, Peptide M, SSL7, Jacalin, and Protein L. The correct choice of purification method depends upon the IgA subclass of the antibody, the species in which it was raised and the intended use of the antibodies. Antibodies from tissue culture supernatant, serum or ascites can be purified using one or more of the following antibody binding proteins offered by InvivoGen.

Peptide M / Agarose - IgA1 and IgA2 purification **NEW**

Peptide M is a 50 aa synthetic peptide derived from a streptococcal M protein containing an additional C-terminal cysteine residue. Peptide M binds monomeric and dimeric human IgA of both subclasses (IgA1 and IgA2) with high specificity and affinity. It also binds bovine IgA but not murine IgA. Peptide M binding occurs at a site in IgA-Fc conserved in human IgA1 and IgA2 and bovine IgA but not in mouse IgA. Peptide M can be used for single-step affinity purification of IgA and for specific detection of antigen-bound IgA¹.

Binding capacity: 6 mg human IgA per ml of gel

SSL7 / Agarose - IgA1 and IgA2 purification

SSL7 (*Staphylococcus aureus* superantigen-like protein 7; formally known as SET1), is a staphylococcus toxin isolated from *Staphylococcus aureus*. SSL7 binds with a high affinity to the monomeric form of human IgA1 and IgA2. SSL7 has no affinity for human IgG, therefore, it can be used to purify human IgA from human sera, milk and other biological samples^{2,3}. SSL7 also binds the secretory form of IgA found in milk from humans, cows, and sheep. SSL7 will bind bovine IgA antibodies present in milk, but not bovine IgA present in serum. SSL7 does not bind mouse, rabbit, sheep or goat IgA present in serum².

Binding capacity: 1 mg human IgA per ml of gel

Jacalin / Agarose - IgA1 purification

Jacalin is an a D-galactose binding lectin extracted from jack-fruit seeds (*Artocarpus integrifolia*). Jacalin immobilized on supports such as agarose is useful for the purification of human serum or secretory IgA^{1,4,5}. IgA can be separated from human IgG and IgM in human serum or colostrums using Immobilized Jacalin. This support is also useful for removing contaminating IgA from IgG samples. Additionally, Jacalin binds IgD⁴. Jacalin can also be used to separate IgA1 subclass from IgA2⁵.

Binding capacity: 1-3 mg human IgA per ml of gel

Protein L / Agarose - κ light chain specific

Protein L is an immunoglobulin-binding protein expressed by the anaerobic bacterial species *Peptostreptococcus magnus*⁶. Protein L binds specifically to the variable domain of Ig κ light chain, as a consequence Protein L has the capacity to purify κ light containing IgA antibodies⁷. It binds strongly to human κ light chain subclasses I, III and IV, and also to most κ light chains of other species such as rat and mouse. As it recognizes κ light chains, protein L can bind to all classes of Ig, in contrast to Protein A and Protein G which interact with the Fc region and bind exclusively to IgG heavy chains. Protein L does not bind bovine immunoglobulins which are present in the fetal bovine serum (FBS) and thus provides a convenient way to purify κ light chain-containing monoclonal antibodies from culture supernatant.

Binding capacity >5 mg of human IgA/IgG per ml of gel

PRODUCT	Human κ light chain	Human λ light chain	Human IgA1	Human IgA2	Human IgG	Human IgM	Human IgE	Human IgD	Mouse IgA	Rat IgA	Bovine IgA
Peptide M	-	-	++++	++++	-	-	n/a	n/a	-	n/a	+
SSL7	-	-	++++	++++	-	-	n/a	-	-	++++	- (serum) + (milk)
Jacalin	-	-	++++	-/+	-	n/a	n/a	+++	-	-	-
Protein L	++++	-	++++	++++	++++	++++	++++	++++	++++	++++	-

1. Sandin C. et al., 2002. Isolation and detection of human IgA using a streptococcal IgA-binding peptide. *J Immunol.* 169(3):1357-64. 2. Langley et al., 2005. The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc alpha RI binding and serum killing of bacteria. *J Immunol* 174 :2926-2933. 3. Ramsland PA. et al., 2007. Structural basis for evasion of IgA immunity by *Staphylococcus aureus* revealed in the complex of SSL7 with Fc of human IgA1. *PNAS* 104:15051-15056. 4. Aucouturier P. et al., 1998. Jacalin, the human IgA1 and IgD precipitating lectin, also binds IgA2 of both allotypes. *J Immunol Methods* 113:185-191. 5. Gregory RL. et al., 1987. Separation of human IgA1 and IgA2 using jacalin-agarose chromatography. *J Immunol Meth* 99:101-106. 6. Björck L., 1988. Protein L: A novel bacterial cell wall protein with affinity for Ig L chains. *J Immunol.* 1988 Feb 15;140(4):1194-7. 7. Nilson BH. et al., 1993. Purification of antibodies using protein L-binding framework structures in the light chain variable domain. *J Immunol Methods.* 164(1):33-40.

PRODUCT	QUANTITY	CAT. CODE
Peptide M / Agarose NEW	2 ml 5 ml	gel-pdm-2 gel-pdm-5
SSL7 / Agarose	2 ml 10 ml	gel-ssl-2 gel-ssl-10
Jacalin / Agarose	2 ml 5 ml	gel-jac-2 gel-jac-5
Protein L / Agarose	2 ml 10 ml	gel-protl-2 gel-protl-10

IgA Detection and Quantification

InvivoGen provides all the reagents necessary to detect and quantify the light and heavy chains of IgA antibodies by sandwich ELISA: capture antibodies, revelation antibodies and IgA standards. InvivoGen offers also a polyclonal rabbit antiserum to human J chain for the detection of dimeric IgA.

IgA κ Light Chain Sandwich ELISA

Goat F(ab')₂ anti-human kappa - Capture antibody

Goat F(ab')₂ anti-human kappa was generated from antibodies isolated from antisera of goats hyperimmunized with human myeloma proteins containing κ light chains. Antibodies were purified by affinity chromatography and digested by pepsin to remove the Fc portion, to avoid non-specific binding through Fc receptors.

Goat F(ab')₂ anti-human kappa allows the capture of κ light chain-containing human IgA antibodies.

Goat anti-human kappa - HRP - Revelation antibody

Goat anti-human kappa-HRP is conjugated with horseradish peroxidase to allow the detection of human IgA kappa light chain by ELISA.

Human IgA kappa - IgA light chain standard

Human IgA kappa is a human IgA κ isotype control purified from human myeloma serum.

Contents and Storage

Goat F(ab')₂ anti-human kappa is provided as 0.5 mg of purified immunoglobulin in 1.0 ml of 100 mM borate buffered saline, pH 8.0.

Goat anti-human kappa-HRP is supplied as 1.0 ml of stock solution in 50% glycerol/50% PBS, pH 7.4.

Human IgA kappa is provided filtered sterile at a concentration of 0.5 mg/ml in 1.0 ml of 100 mM borate buffered saline, pH 8.0.

Products are shipped at room temperature and should be stored at 4°C.

PRODUCT	QTY	CAT. CODE
Goat F(ab')₂ anti-human kappa	0.5 mg	fab-igak
Goat anti-human kappa - HRP	1 ml	hrp-igak
Human IgA kappa	0.5 mg	ctrl-igak

IgA Heavy Chain Sandwich ELISA

Goat F(ab')₂ anti-human IgA - Capture antibody

Goat F(ab')₂ anti-human IgA was generated from antibodies isolated from antisera of goats hyperimmunized with human IgA paraproteins. Antibodies were purified by affinity chromatography and digested by pepsin to remove the Fc portion, to avoid non-specific binding through Fc receptors.

Goat F(ab')₂ anti-human IgA allows the capture of the heavy chain of human IgA antibodies.

Goat anti-human IgA - HRP - Revelation antibody

Goat anti-human IgA-HRP is conjugated with horseradish peroxidase to allow the detection of human IgA heavy chain by ELISA.

Human IgA from colostrum - IgA standard

Human IgA is isolated from pooled normal human colostrum by fractionation and ion-exchange chromatography.

Contents and Storage

Goat F(ab')₂ anti-human IgA is provided as 0.5 mg of purified immunoglobulin in 1.0 ml of 100 mM borate buffered saline, pH 8.0.

Goat anti-human IgA-HRP is supplied as 1.0 ml of stock solution in 50% glycerol/50% PBS, pH 7.4.

Human IgA is supplied as an essentially salt-free, lyophilized powder.

Products are shipped at room temperature and should be stored at 4°C.

PRODUCT	QTY	CAT. CODE
Goat F(ab')₂ anti-human IgA	0.5 mg	fab-iga
Goat anti-human IgA - HRP	1 ml	hrp-iga
Human IgA	0.5 mg	ctrl-iga

J Chain Antiserum

J chain antiserum was prepared by injection of purified human J chain in rabbits. Human J chain is distinct from all other chain components of polymeric IgA. J chain antiserum can be used for the detection of dimeric IgA by Western blotting (working dilution 1:1,000).

Contents and Storage

J chain antiserum is provided as 100 μ g lyophilized antiserum. J chain antiserum is sterile and azide-free. Product is shipped at room temperature and should be stored at -20°C or 4°C once resuspended.

PRODUCT	QTY	CAT. CODE
J Chain Antiserum	100 μ g	pab-jc