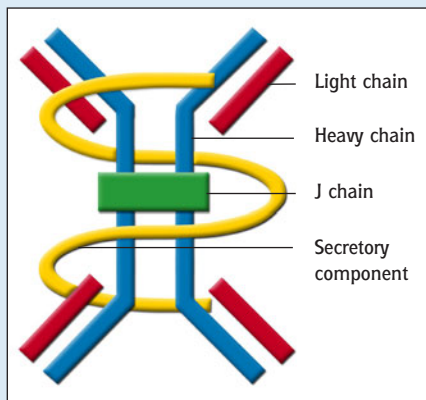


InvivoGen Insight

Immunoglobulin A : Antibodies for Research & Therapeutic Applications

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 functions^{2, 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 defense 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-1^{4,6}.

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 1 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 (described in this newsletter). 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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2. **Woof JM. & Kerr MA., 2007.** The function of immunoglobulin A in immunity. *J Pathol.* 208(2):270-82. Review.
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.

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Products

Immunoglobulin A

- ❖ Cytokine Neutralizing IgA MAbs
- ❖ TLR Neutralizing IgA MAbs
- ❖ IgA Detection Abs

Cytokines

- ❖ HEK-Blue™ Cytokine Cells
- ❖ Recombinant Cytokines

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Neutralizing IgA Antibodies

InvivoGen provides monoclonal antibodies (MAbs) of the IgA isotype specifically isolated for their ability to neutralize key cytokines of the innate immune response. The neutralizing activity of these IgA MAbs were determined using HEK-Blue™ Cytokine Cells. Cytokine stimulation assays were performed in the absence or presence of the IgA MAbs and monitored using QUANTI-Blue™ (see below).

HEK-Blue™ Cytokine Cells

- Monitor the activation of signaling pathways induced by key cytokines
- Detect biologically active cytokines in samples
- Screen or validate neutralizing molecules such as antibodies and siRNA/shRNA

Description

HEK-Blue™ Cytokine Cells is a new family of engineered cell lines designed to provide a simple, rapid and reliable method to monitor the activation of signaling pathways induced by key cytokines. They allow to detect these biologically active cytokines and can also be used to screen for compounds exhibiting agonist and antagonist activities. HEK-Blue™ Cytokines Cells are engineered HEK293 cells featuring the HEK-Blue™ technology. This technology is based on the inducible expression of an optimized secreted embryonic alkaline phosphatase (SEAP) reporter gene and its quantitative detection using QUANTI-Blue™, a SEAP colorimetric detection medium. HEK-Blue™ Cells express the SEAP reporter gene under the control of different inducible promoters chosen or developed to be selectively activated by the cytokines known to induce the pathway of interest. In the presence of a cytokine, agonist or antagonist compound, the pathway is activated or inhibited modulating the SEAP activity. The amount of SEAP secreted in the cell supernatant can be measured spectrometrically at 650 nm.

HEK-Blue™ IFN- α / β Cells

- Monitor the JAK/STAT/ISGF3 pathway
- Detection range for IFN α : 1 - 10⁴ IU/ml
- Detection range for IFN β : 10 - 10⁴ IU/ml

HEK-Blue™ IFN- α / β Cells allow the detection of bioactive human type I IFNs by monitoring the activation of the ISGF3 pathway. These cells were generated by stable transfection of HEK293 cells with the human STAT2 and IRF9 genes to obtain a fully active type I IFN signaling pathway. They were further transfected with an engineered SEAP reporter gene under the control of the IFN- α / β -inducible ISG54 promoter. Secretion of SEAP is specifically induced by type I IFNs. Stimulation of HEK-Blue™ IFN- α / β Cells with selected cytokines has no effect on SEAP expression.

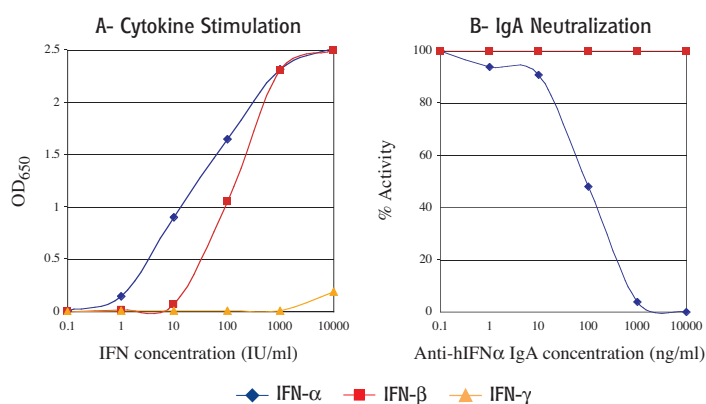
Stimulation of HEK-Blue™ IFN- α / β Cells with IFN- α can be blocked by the neutralizing monoclonal hIFN- α -IgA antibody. Thus HEK-Blue™ IFN- α / β cells can be used as IFN β -specific sensor cells by blocking IFN- α stimulation with hIFN- α -IgA.

HEK-Blue™ IL-6 Cells

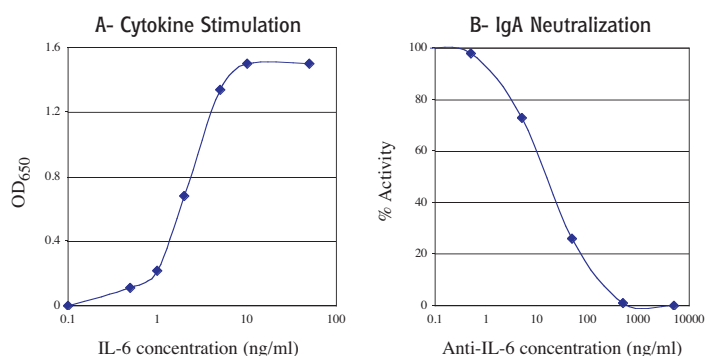
- Monitor the JAK/STAT-3 pathway
- Detection range for IL-6: 0.5 - 50 ng/ml

HEK-Blue™ IL-6 Cells allow the detection of bioactive human IL-6 by monitoring the activation of the STAT-3 pathway. These cells were generated by stable transfection of HEK293 cells with the human IL-6R gene. They were further transfected with an engineered SEAP reporter gene under the control of the IFN β minimal promoter fused to four STAT3 binding sites. HEK-Blue™ IL-6 Cells secrete SEAP following stimulation by IL-6 and no other cytokines.

Stimulation of HEK-Blue™ IL-6 Cells with IL-6 can be blocked by the neutralizing monoclonal hIL-6-IgA antibody.



A- Stimulation of HEK-Blue™ IFN- α / β cells by recombinant human IFN α 2b, IFN β 1a and IFN γ was assessed by measuring the levels of SEAP secreted in the supernatant using QUANTI-Blue™. **B-** HEK-Blue™ IFN- α / β cells were used to determine the neutralization activity of anti-human IFN α . Approximately 100 ng/ml of the antibody neutralizes 50% of the bioactivity due to 10³ IU/ml of IFN α 2b. Anti-human IFN α has no neutralizing activity on IFN β .



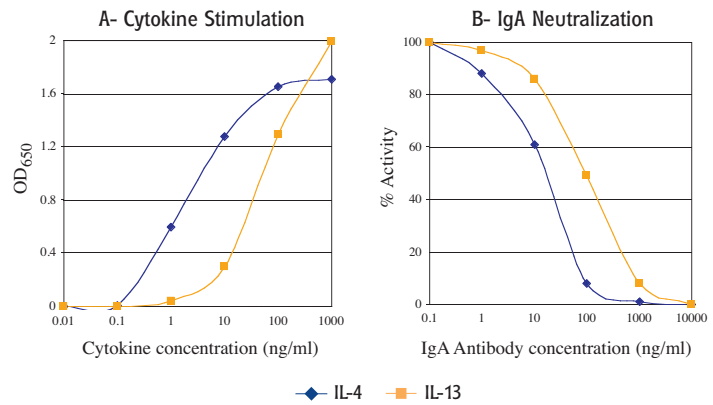
A- Stimulation of HEK-Blue™ IL-6 cells by recombinant human IL-6 was assessed by measuring the levels of SEAP secreted in the supernatant using QUANTI-Blue™. **B-** HEK-Blue™ IL-6 cells were used to determine the neutralization activity of hIL-6-IgA. Approximately 20 ng/ml of the antibody neutralizes 50% of the bioactivity due to 5 ng/ml of IL-6.

HEK-Blue™ IL-4/IL-13 Cells

- Monitor the JAK/STAT-6 pathway
- Detection range for IL-4: 0.5 - 500 ng/ml
- Detection range for IL-13: 5 - 1000 ng/ml

HEK-Blue™ IL-4/IL-13 Cells allow the detection of bioactive human IL-4 and IL-13 by monitoring the activation of the STAT-6 pathway. These cells were generated by stable transfection of HEK293 cells with the human STAT6 gene to obtain a fully active STAT6 pathway. They were further transfected with an engineered SEAP reporter gene under the control of the IFN β minimal promoter fused to four STAT6 binding sites. HEK-Blue™ IL-4/IL-13 Cells produce SEAP in response to IL-4 or IL-13 stimulation and to a lower extent IFN α which can also activate STAT6.

Stimulation of HEK-Blue™ IL-4/IL-13 Cells with IL-4 or IL-13 can be blocked by the neutralizing monoclonal hIL-4-IgA and hIL-13-IgA antibodies. Thus HEK-Blue™ IL-4/IL-13 cells can become IL-4 or IL-13-specific sensor cells by blocking either IL-13 or IL-4 stimulation with hIL-13-IgA or hIL-4-IgA, respectively.



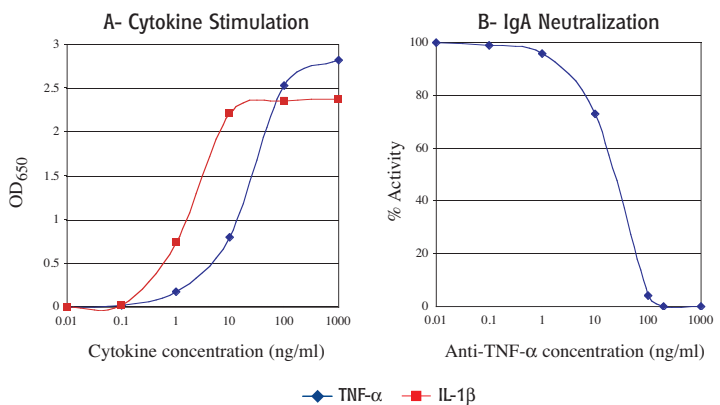
A- Stimulation of HEK-Blue™ IL-4/IL-13 cells by recombinant human IL-4 and IL-13 was assessed by measuring the levels of SEAP secreted in the supernatant using QUANTI-Blue™. **B-** HEK-Blue™ IL-4/IL-13 cells were used to determine the neutralization activity of hIL-4-IgA and hIL-13-IgA. Approximately 20 ng/ml of the anti-IL-4 or 100 ng/ml of the anti-IL-13 neutralizes 50% of the bioactivity due to 1 ng/ml of IL-4 or 50 ng/ml of IL-13, respectively.

HEK-Blue™ TNF- α /IL-1 β Cells NEW

- Monitor the NF- κ B and AP-1 pathways
- Detection range for TNF- α : 0.5 - 500 ng/ml
- Detection range for IL-1 β : 0.2 - 20 ng/ml

HEK-Blue™ TNF- α /IL-1 β Cells are designed to detect human bioactive TNF- α and IL-1 β by monitoring the activation of the NF- κ B and AP-1 pathways. These cells were generated by stable transfection of HEK293 cells with an engineered SEAP reporter gene under the control of the IFN β minimal promoter fused to five NF- κ B and five AP-1 binding sites. Secretion of SEAP by HEK-Blue™ TNF- α /IL-1 β Cells is specifically induced by TNF α and IL-1 β .

Stimulation of HEK-Blue™ TNF- α /IL-1 β Cells with TNF- α can be blocked by the neutralizing monoclonal hTNF- α -IgA antibody. Thus HEK-Blue™ TNF- α /IL-1 β can be used as IL-1 β -specific sensor cells by blocking TNF- α stimulation with hTNF- α -IgA.



A- Stimulation of HEK-Blue™ TNF- α /IL-1 β cells by recombinant human TNF- α and IL-1 β was assessed by measuring the levels of SEAP secreted in the supernatant using QUANTI-Blue™. **B-** HEK-Blue™ TNF- α /IL-1 β cells were used to determine the neutralization activity of hTNF- α -IgA. Approximately 20 ng/ml of the antibody neutralizes 50% of the bioactivity due to 20 ng/ml of TNF- α .

Contents and Storage

HEK-Blue™ Cytokine Cells are grown in DMEM medium with 10% FBS supplemented with selective antibiotics. HEK-Blue™ TNF- α /IL-1 β Cells are resistant to Zeocin™, HEK-Blue™ IFN- α / β Cells and HEK-Blue™ IL-4/IL-13 Cells are resistant to Zeocin™ and blasticidin. HEK-Blue™ IL-6 Cells are resistant to Zeocin™ and hygromycin. Each vial contains 3-5 x 10⁶ cells and supplied with 100 μ l of each of the antibiotics and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice.

Product	Quantity	Cat. Code
HEK-Blue™-Blue IFN α / β Cells	3-5 x 10 ⁶ cells	hkb-ifnab
HEK-Blue™-Blue IL6 Cells	3-5 x 10 ⁶ cells	hkb-il6
HEK-Blue™-Blue IL-4/IL-13 Cells	3-5 x 10 ⁶ cells	hkb-stat6
HEK-Blue™-Blue TNF α /IL-1 β Cells	3-5 x 10 ⁶ cells	hkb-tnfil1

Related Products

- Neutralizing IgA Antibodies (see next page)
- Recombinant Cytokines

InvivoGen provides a selection of recombinant human cytokines that are fully proficient in inducing their cognate signaling pathway in HEK-Blue™ Cytokine Cells. These recombinant cytokines are provided as sterile lyophilized powder, endotoxin-free. Products are shipped at room temperature.

Product	Quantity	Cat. Code
Interferon β 1a	2 μ g (5 x 10 ⁵ IU)	hifn-b1a
Interferon γ	20 μ g (4 x 10 ⁵ IU)	hifn-g
Interleukin 1 β	10 μ g	hil-1b
Interleukin 4	10 μ g	hil-4
Interleukin 6	10 μ g	hil-6
Interleukin 13	2 μ g	hil-13
Tumor Necrosis Factor α	20 μ g	htnf-a1a

Neutralizing IgA Antibodies

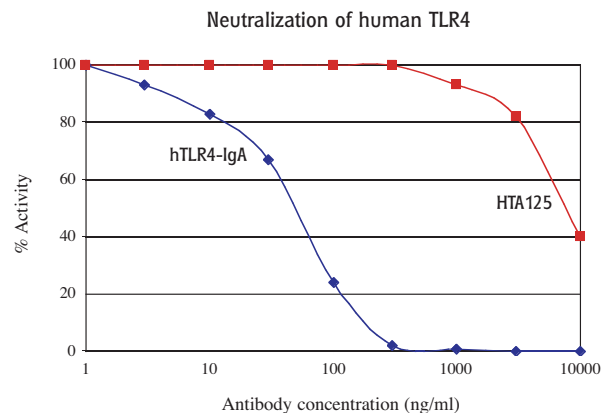
NEW

Description

Our neutralizing IgA antibodies are chimeric monoclonal 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.

Content 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.



HEK-Blue™ TLR4 cells were used to compare the neutralization activity of hTLR4-IgA and HTA125, a monoclonal IgG. Approximately 50 ng/ml of hTLR4-IgA and 8 µg/ml HTA125 neutralize 50% of the bioactivity due to 10 ng/ml of LPS.

Antigen	Product	Reactivity	Neutralization (ND50)	Quantity	Cat. Code
Cytokines					
Human Interferon α	hIFNα-IgA	Human	100 ng/ml for 1000 U/ml rhIFNα	100 µg	maba-hifna
Human Interleukin 4	hIL-4-IgA	Human	20 ng/ml for 1 ng/ml rhIL-4	100 µg	maba-hil4
Human Interleukin 6	hIL-6-IgA	Human	20 ng/ml for 5 ng/ml rhIL-6	100 µg	maba-hil6
Human Interleukin 13	hIL-13-IgA	Human	100 ng/ml for 50 ng/ml rhIL-13	100 µg	maba-hil13
Human Tumor Necrosis Factor α	hTNFα-IgA	Human	20 ng/ml for 20 ng/ml rhTNFα	100 µg	maba-htnfa
Toll-Like Receptors					
Human Toll-Like Receptor 2	hTLR2-IgA	Human	0.1-0.5 µg/ml for 10 ng/ml FSL-1	100 µg	maba-htlr2
Human Toll-Like Receptor 4	hTLR4-IgA	Human	50 ng/ml for 10 ng/ml LPS	100 µg	maba-htlr4
Human Toll-Like Receptor 5	hTLR5-IgA	Human	10 ng/ml for 1 ng/ml flagellin	100 µg	maba-htlr5
Control					
Human IgA2	Control-IgA	Human	None	100 µg	maba-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	Quantity	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