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 surface. 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.

IgA 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. 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 FcγR1 triggering antibody-dependent-cell-mediated cytotoxicity (ADCC). Due to their specific effector functions, IgA may 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.

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 cells. 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 Ctr, obtained through insertion of the human α1 gene in place of the switch sequence Sμ, 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 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.


---

**Inside this issue:**

**Products**

**Immunoglobulin A**
- Cytokine Neutralizing IgA MAbs
- TLR Neutralizing IgA MAbs
- IgA Detection Abs

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

---

**InvivoGen Insight**

**HEK-Blue™ Cytokine Cells**

**InvivoGen**

3950-A Sorrento Valley Blvd
San Diego, CA 92121 USA
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™ Cytokine 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 the JAK/STAT/ISGF3 pathway. These cells were further transfected with an engineered 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 IFNα: 1 - 10⁴ IU/ml
- Detection range for IFNβ: 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-α-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 IFNβ 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.
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 STAT6 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 IFNβ 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 IFNα 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.

HEK-Blue™ TNF-α/IL-1β Cells

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

HEK-Blue™ TNF-α/IL-1β Cells are designed to detect human bioactive TNF-α and IL-1β by monitoring the activation of the NF-kB 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 IFNβ minimal promoter fused to five NF-kB and five AP-1 binding sites. Secretion of SEAP by HEK-Blue™ TNF-α/IL-1β Cells is specifically induced by TNFα and IL-1β.

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

Contents and Storage

HEK-Blue™ Cytokine Cells are grown in DMEM medium with 10% FBS supplemented with selective antibiotics. HEK-Blue™ TNF-α/IL-1β 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 µl of each of the antibiotics and 1 pouch of endotoxin-free. Products are shipped at room temperature.

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.
Neutralizing IgA Antibodies

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.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Product</th>
<th>Reactivity</th>
<th>Neutralization (ND50)</th>
<th>Quantity</th>
<th>Cat. Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Interferon α</td>
<td>hIFNα-IgA</td>
<td>Human</td>
<td>100 ng/ml for 1000 U/ml rhIFNα</td>
<td>100 µg</td>
<td>maba-hifna</td>
</tr>
<tr>
<td>Human Interleukin 4</td>
<td>hIL-4-IgA</td>
<td>Human</td>
<td>20 ng/ml for 1 ng/ml rhIL-4</td>
<td>100 µg</td>
<td>maba-hil4</td>
</tr>
<tr>
<td>Human Interleukin 6</td>
<td>hIL-6-IgA</td>
<td>Human</td>
<td>20 ng/ml for 5 ng/ml rhIL-6</td>
<td>100 µg</td>
<td>maba-hil6</td>
</tr>
<tr>
<td>Human Interleukin 13</td>
<td>hIL-13-IgA</td>
<td>Human</td>
<td>100 ng/ml for 50 ng/ml rhIL-13</td>
<td>100 µg</td>
<td>maba-hil13</td>
</tr>
<tr>
<td>Human Tumor Necrosis Factor α</td>
<td>hTNFα-IgA</td>
<td>Human</td>
<td>20 ng/ml for 20 ng/ml rhTNFα</td>
<td>100 µg</td>
<td>maba-htnfa</td>
</tr>
<tr>
<td>Toll-Like Receptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Toll-Like Receptor 2</td>
<td>hTLR2-IgA</td>
<td>Human</td>
<td>0.1-0.5 µg/ml for 10 ng/ml FSL-1</td>
<td>100 µg</td>
<td>maba-htrl2</td>
</tr>
<tr>
<td>Human Toll-Like Receptor 4</td>
<td>hTLR4-IgA</td>
<td>Human</td>
<td>50 ng/ml for 10 ng/ml LPS</td>
<td>100 µg</td>
<td>maba-htrl4</td>
</tr>
<tr>
<td>Human Toll-Like Receptor 5</td>
<td>hTLR5-IgA</td>
<td>Human</td>
<td>10 ng/ml for 1 ng/ml flagellin</td>
<td>100 µg</td>
<td>maba-htrl5</td>
</tr>
<tr>
<td>Control</td>
<td>Control-IgA</td>
<td>Human</td>
<td>None</td>
<td>100 µg</td>
<td>maba-ctrl</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat F(ab')2 Anti-Human IgA - Biotin</td>
<td>500 µg</td>
<td>chiga-biot</td>
</tr>
<tr>
<td>Goat F(ab')2 Anti-Human IgA - FITC</td>
<td>500 µg</td>
<td>chiga-fitc</td>
</tr>
<tr>
<td>Goat F(ab')2 IgG Isotype Control - FITC</td>
<td>100 tests</td>
<td>cgg-fitc</td>
</tr>
</tbody>
</table>