Mouse sIgA/dimeric IgA ELISA kit

Catalog No.E0641m
(96 tests)
Operating instruction

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Intended use
This immunoassay kit allows for the specific measurement of Mouse secretory Immunoglobulin A, sIgA (dimeric IgA) concentrations in cell culture supernates, saliva, Breast Milk.

Introduction
Immunoglobulin A (IgA) is an antibody and, in its secretory form, is the main immunoglobulin found in mucous secretions, including tears, saliva, colostrum, intestinal juice, vaginal fluid and secretions from the prostate and respiratory epithelium. It is also found in small amounts in blood. Because it is resistant to degradation by enzymes, secretory IgA can survive in harsh environments such as the digestive and respiratory tracts, to provide protection against microbes that multiply in body secretions. IgA does not activate complement, and opsonises only weakly. Its heavy chains are of the type α.

IgA is found in secretions in a specific form called secretory IgA, polymers of 2 IgA monomers linked by two additional chains. One of these is the J chain (joining chain), which is a polypeptide of molecular mass 1.5 kD, rich with cysteine and structurally completely different from other immunoglobulin chains. This chain is formed in the IgA-secreting cells. The high prevalence of IgA in mucosal areas is a result of a cooperation between plasma cells that produce polymeric IgA (plgA), and mucosal epithelial cells that express the an immunoglobulin receptor called the polymeric Ig receptor (plgR). plgA is released from the nearby activated plasma cells and binds to plgR. This results in transportation of IgA across mucosal epithelial cells and its cleavage from plgR for release into external secretions.
Mouse Immunoglobulin A (IgA)is primarily secreted across the mucosal tract into the stomach and intestines. This prevents microbes from binding to epithelial cells in the digestive and respiratory tracts. Mouse Immunoglobulin A (IgA)helps to fight against pathogens that contact the body surface, are ingested, or are inhaled.

Test principle
This assay employs the competitive inhibition enzyme immunoassay technique. A polyclonal antibody specific for slgA has been pre-coated onto a microplate. A competitive inhibition reaction is launched between HRP labeled slgA and unlabeled slgA (Standards or samples) with the pre-coated antibody specific for slgA. The more the amount of slgA in samples, the less the HRP labeled slgA bound by pre-coated antibody. The substrate solution are added to the wells, respectively. And the color develops in opposite to the amount of slgA bound in the initial step. The color development is stopped and the intensity of the color is measured.
Materials and components

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay plate</td>
<td>1</td>
</tr>
<tr>
<td>Standard</td>
<td>2</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>Assay Diluent A</td>
<td>2 x 10ml</td>
</tr>
<tr>
<td>Detection Reagent A</td>
<td>2 x 120ul</td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>1 x 30ml</td>
</tr>
<tr>
<td>(25 x concentrate)</td>
<td></td>
</tr>
<tr>
<td>Substrate</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 10ml</td>
</tr>
</tbody>
</table>

Sample collection and storage

**Cell culture supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at \( \leq -20^\circ C \). Avoid repeated freeze-thaw cycles.

**Saliva** - Collect Saliva using a collection device such as an equivalent. assay immediately or aliquot and store samples at \( \leq -20^\circ C \). Avoid repeated freeze-thaw cycles.

**Breast Milk** - Centrifuge for 15 minutes at 10,000 x g at 2 - 8\(^{\circ}\) C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at -20\(^{\circ}\) C. Avoid repeated freeze-thaw cycles.

**Note:** Citrate plasma has not been validated for use in this assay. Saliva Collector must not have any protein binding or filtering capabilities.

Limitations of the procedure

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1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the QuantiKine Immunoassay, the possibility of interference cannot be excluded.

Reagent preparation

**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

**Standard** - Reconstitute the **Standard** with 1.0 mL of **Sample Diluent**. This reconstitution produces a stock solution of 10 ug/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard
(10 ug/ml). The Sample Diluent serves as the zero standard (0 ug/ml).

**Detection Reagent A** - Dilute to the working concentration specified on the vial label using **Assay Diluent A** (1:100), respectively.

**Assay procedure**

Allow all reagents to reach room temperature. Arrange and label required number of strips.

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Pipette 50uL of the reference standard or Control, or sample* per tube and then add 50 uL of Detection Reagent A to each tube. Mix each tube thoroughly. Add 100ul the mixed solution per well and incubate for 1 hour at 37°C. **Detection Reagent A** may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
3. Aspirate each well and wash, repeating the process three times for a total of five washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher for 5 times. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 90 uL of **Substrate Solution** to each well. Incubate for 30 minutes at 37°C. Protect from light.
5. Add 50 uL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

**Specificity**

This assay recognizes recombinant and natural Mouse dimeric IgA (sIgA). No significant cross-reactivity or interference was observed.

**Sensitivity**

The minimum detectable dose of Mouse dimeric IgA (sIgA) is typically less than 0.05 ug/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.

**Detection Range**

0.156-10 ug/ml. The assay range was estimated by calculating the coefficient of variation (CV) of each standard constructing five independent standard curves. The standard curve concentrations used for the ELISA’s were 10 ug/ml, 5 ug/ml, 2.5 ug/ml, 1.25 ug/ml, 0.625 ug/ml, 0.325 ug/ml, 0.156 ug/ml.

**Important Note:**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.

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3. Duplication of all standards and specimens, although not required, is recommended.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

**Calculation of results**
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the sIgA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**Storage of test kits and instrumentation**
1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

**Precaution**
The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.