Secretory IgA (sIgA) ELISA

Catalog Number:  SGA35-K01
96 Wells
For Research Use Only. Not for use in diagnostic procedures.
1. Intended use

The Eagle Biosciences Secretory IgA (sIgA) ELISA Kit is intended for the quantitative determination of sIgA in stool and saliva. This Secretory IgA (sIgA) ELISA Kit is for Research Use Only and not be used for diagnostic procedures.

2. Introduction

Secretory IgA is comprised of two immunoglobulin A molecules, which are joined by a J-protein and a secretory component. The secretory component is synthesized by epithelial cells of the mucous membrane of gastrointestinal, respiratory and urogenital tracts. Secretory IgA is also produced by the saliva, tear and mammary glands. The plasma cells in the subendothelial area of mucous membranes are releasing a complex of two IgA-molecules, which are joined over the J-protein. This complex is binding to a secretory component, located at the surface of the epithelial cell. After binding, the sIgA is transported across the cell and excreted by exocytosis.

The determination of secretory IgA (sIgA) allows a first overview of the functionality of the gastrointestinal associated immune system (GALT). At this the secretory power and the degree of stimulation of the plasma cells of the intestinal submucosa is determined.

The Eagle Biosciences Secretory IgA (sIgA) ELISA kit allows for an easy, rapid and precise quantitative determination of secretory IgA in biological samples. The kit includes all reagents ready to use for preparation of the samples.

3. Warnings and precautions

- All reagents of this kit are strictly intended for Research Use Only.
- Do not interchange kit components from different lots.
- The stop solution (STOP) contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.
- The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill should be wiped out immediately with copious quantities of water.
- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- The reagents of the Secretory IgA (sIgA) ELISA kit contain bactericides to protect against bacterial growth. Avoid the contact with the skin or mucous membrane.
- Reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

**4. Materials Provided**

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Component</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC6100mtp</td>
<td>MTP</td>
<td>Microtiter plate coated</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>IC6100wp</td>
<td>WASHBUF</td>
<td>ELISA wash buffer conc. 10 fold</td>
<td>100 ml</td>
</tr>
<tr>
<td>IC6100st</td>
<td>STD</td>
<td>Standard (1 ml) (0; 22.2; 66.6; 200; 600)</td>
<td>5 vials</td>
</tr>
<tr>
<td>IC6100ko</td>
<td>CTRL</td>
<td>Control 1 and 2 (1 ml)</td>
<td>1 vial each</td>
</tr>
<tr>
<td>IC6100kg</td>
<td>CONJ</td>
<td>Conjugate, peroxidase labeled antibody</td>
<td>15 ml</td>
</tr>
<tr>
<td>IC6100su</td>
<td>SUB</td>
<td>TMB substrate (tetramethylbenzidine)</td>
<td>15 ml</td>
</tr>
<tr>
<td>IC6100sp</td>
<td>STOP</td>
<td>Stop solution</td>
<td>7 ml</td>
</tr>
</tbody>
</table>

**5. Additional special equipment**

- Laboratory balance
- Centrifuge, 3000xg
- Glass or plastic vials
- Various pipettes
- Foil to cover the microtiter plate
- Multichannel or multi-pipette
- ELISA reader with filter 450 nm (reference filter 620 or 690 nm)
- Microtiter plate shaker
- Vortex mixer

**6. Reagent preparation**

**Microtiter Plate (MTP):** Take the needed strips out of the bag and mount them on the holder. Please take care that the package has reached room temperature before opening the bag. Strips which are not needed could be stored at 2-8°C. Please dispose the holder when all strips are used.
**Wash buffer (WASHBUF):** Dilute the wash buffer concentrate 1:10 with deionized or distilled water (1 part buffer + 9 parts water). The dilution is stable for 14 days at 2-8°C. *Important: When storing the wash buffer concentrate at 2-8°C crystallization could occur. Before dilution all crystals must be dissolved.*

It is recommended to dilute only the amount of buffer which is used to process the given samples. All other test reagents are stable at 2-8 °C, up to the date of expiry stated on the label.

### 7. Specimen

#### Stool samples
sIgA is extracted by the sample dilution buffer out of the stool sample.

- 100 mg stool are mixed with 5 ml WASHBUF on a vortex mixer until the mixture is homogenous.
- 1 ml of the mixture is transferred into an “Eppendorf” reaction vial and centrifuged for 10 min at 10000xg.
- Dilute the supernatant 1:250 with WASHBUF (4 µl + 996 µl WASHBUF)
- 100 µl of the dilution are used in the test per well.

#### Saliva samples
To get a good comparability between different samples, we recommend to take the samples at the same time. The patient should not eat or drink 30 min before taking the sample. The sample should be stored and shipped on ice.

- The sample is centrifuged for 10 min at 3000xg. The centrifugation will create a sediment, a liquid phase and a foamy supernatant.
- Dilute the liquid phase 1:10000 with WASHBUF
  - Dilution A: 10 µl + 990 µl WASHBUF
  - Dilution B: 10 µl + 990 µl WASHBUF
- 100 µl of the dilution B are used in the test per well.

### 8. Procedure

#### Principle of the Assay
The Secretory IgA (sIgA) ELISA kit determines human secretory IgA according to the “sandwich”-principle. sIgA in sample, standard and controls binds to antibodies, which are coated to the microtiter plate. After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop.
solution. The optical densities are read at 450 nm (against the reference wavelength 620 nm) in a microtiter plate reader. The sIgA concentration can be calculated from the standard curve.

Sample preparation
All reagents and samples should be at room temperature (18-26°C) and mixed well before use. The position of standards, controls and samples should be noted.

1. Washing step
   - Take out the needed strips of the microtiter plate and wash 1x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the washing step.

2. Incubation samples
   - Pipette 100 µl STD, CTRL and samples in duplicate in the microtiter plate.
   - The strips are covered and incubated by shaking for 60 min at room temperature (18-26 °C).
   - The reaction in each well starts immediately. Pipetting should be performed as quickly as possible. When processing many samples at once the samples should be pipetted to a separate microtiter plate (150 µl) and transferred simultaneously using a multichannel pipette.

3. Washing step
   - Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

4. Incubation conjugate
   - Pipette 100 µl CONJ in each microwell.
   - The strips are covered and incubated by shaking for 60 min at room temperature (18-26 °C).

5. Washing step
   - Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

6. Incubation substrate
   - Pipette 100 µl SUB in each microwell.
   - Incubate for 10-15 min at room temperature (18-26 °C) in the dark.

7. Stopping reaction
   - Pipette 50 µl STOP in each microwell, mix well.

8. Reading
• Read the absorbance at 450 nm. If the microtiter plate reader allows to use a reference wavelength use 620 or 690 nm as reference wavelength.
• Reading should be done within 5 min after stopping reaction.
• In case that the highest standard exceeds the range of the reader the reading should be done at 405 nm against 620 nm (690 nm).

9. Calculation of analytical results

For calculating the results we recommend to use the 4-parameter algorithm. Is this algorithm not available a “point to point” or a “spline” function can be used.

Stool samples
• The obtained sIgA concentration is multiplied by 12.5
• Dilution 1: 100 mg in 5 ml corresponds to a factor 50 (assumption: 1 g stool = 1 ml)
• Dilution 2: Factor 250

Calculation: Conc. Patient [µg/ml] = obtained conc. [ng/ml] x 50 x 250 / 1000

Saliva samples
The obtained sIgA concentration [ng/ml] is multiplied by 10 to get calculated concentration in µg/ml.

Typical Standard Curve

The curve given above is only for demonstration. It must not be used for calculation of your samples.
10. Internal quality control

Reference values

Stool: 510 - 2040 µg/ml
Saliva: 102 – 478 µg/ml

Ref: M. Martin (Hrsg.). Gastroenterologische Aspekte in der Naturheilkunde
ISBN 3-930620-29-4; S.31

We recommend that each laboratory develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. Validation data

Precision and reproducibility

Intra-assay CV:
5.4 % (224.4 ng/ml) [n = 10]
4.5 % (111.9 ng/ml) [n = 10]
6.3 % (33.4 ng/ml) [n = 10]

Inter-assay CV:
6.0 % (227.4 ng/ml) [n = 10]
5.0 % (108.4 ng/ml) [n = 10]
8.2 % (31.8 ng/ml) [n = 10]

Linearity

The dilution of the samples was done with WASHBUF.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution factor</th>
<th>Expected [ng/ml]</th>
<th>Measured [ng/ml]</th>
<th>Recovery [%]</th>
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<tbody>
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<td>1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>122,5</td>
<td>120,5</td>
<td>98,4</td>
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<tr>
<td></td>
<td>1:4</td>
<td>61,3</td>
<td>55,6</td>
<td>90,8</td>
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<td>1:8</td>
<td>30,6</td>
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<td>1:16</td>
<td>15,3</td>
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<td>2</td>
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<td>--</td>
<td>124,5</td>
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<td></td>
<td>1:2</td>
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<td>59,4</td>
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<td>1:4</td>
<td>10,3</td>
<td>8,5</td>
<td>82,5</td>
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</table>
Detection limit

3.1 ng/ml

For the determination of the detection limit 20 replicates of the standard 0 were measured. After addition of the twofold standard deviation to the mean value the concentration was read from the standard curve.

Recovery

<table>
<thead>
<tr>
<th>Sample</th>
<th>Endogen [ng/ml]</th>
<th>Added</th>
<th>Expected [ng/ml]</th>
<th>Measured [ng/ml]</th>
<th>Recovery [%]</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>31.5</td>
<td>22.2</td>
<td>53.7</td>
<td>56.9</td>
<td>106.0</td>
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<td>66.6</td>
<td>98.1</td>
<td>105.8</td>
<td>107.8</td>
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<td></td>
<td>200</td>
<td>231.5</td>
<td>278.6</td>
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<td>120.3</td>
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<tr>
<td>2</td>
<td>112.4</td>
<td>22.2</td>
<td>134.6</td>
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<tr>
<td></td>
<td>66.6</td>
<td>179.0</td>
<td>180.9</td>
<td>101.1</td>
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<td>200</td>
<td>312.4</td>
<td>335.7</td>
<td>107.5</td>
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<tr>
<td>3</td>
<td>248.9</td>
<td>22.2</td>
<td>271.1</td>
<td>279.4</td>
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<td>315.5</td>
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<td>200</td>
<td>448.9</td>
<td>484.1</td>
<td>107.8</td>
<td></td>
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</tbody>
</table>

Cross reactivity

Cross reactivity to other plasma proteins could not be detected in stool and saliva samples.
12. Limitations of the method

Stool and saliva samples with sIgA concentrations above the standard curve should be diluted with wash buffer (WASHBUF) and measured again. In case of strong diarrhea it is possible that even patients with an intact gut associated immune system show lowered values.

13. Disposal

The substrate (SUB) must be disposed as non-halogenated solvent. The stop solution (STOPP) could be neutralized with NaOH and if the pH value is neutral it can be disposed as salt solution. (Important: Reaction will produce heat, be careful)
Please refer to the appropriate national guidelines.

14. Literature references

M. Martin (Hrsg.). Gastroenterologische Aspekte in der Naturheilkunde
ISBN 3-930620-29-4

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.