Secretory IgA ELISA

For the quantitative determination of sIgA in human stool and saliva.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 30-SECHU-E01
Size: 96 Wells
INTENDED USE

The ALPCO Secretory IgA ELISA is intended for the quantitative determination of sIgA in human stool and saliva samples. For Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

This Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the quantitative determination of secretory IgA in stool and saliva. In a first incubation step, the sIgA in the samples is bound to polyclonal antibodies (rabbit anti human IgA), which are immobilized to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled conjugate (mouse anti-sIgA) is added which recognizes specifically the bound secretory IgA. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of secretory IgA.

MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretory IgA Microplate (96 wells)</td>
<td>12 x 8 strips</td>
<td>Ready to use</td>
</tr>
<tr>
<td>Standards (1-5) (0, 22.2, 66.6, 200, 600 ng/mL)</td>
<td>2 x 5 vials</td>
<td>Lyophilized</td>
</tr>
<tr>
<td>Control Levels 1 and 2</td>
<td>2 x 1 vial each</td>
<td>Lyophilized</td>
</tr>
<tr>
<td>Wash Buffer Concentrate</td>
<td>2 x 100 mL</td>
<td>10X</td>
</tr>
<tr>
<td>Extraction Buffer Concentrate</td>
<td>2 x 100 mL</td>
<td>2.5X</td>
</tr>
<tr>
<td>Conjugate Concentrate</td>
<td>1 x 200 µL</td>
<td>101X</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>1 x 15 mL</td>
<td>Ready to use</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 15 mL</td>
<td>Ready to use</td>
</tr>
</tbody>
</table>
MATERIALS REQUIRED

- Precision pipettes for dispensing up to 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled/Deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 700-900 rpm
- Microplate reader
- Centrifuge (1,000 x g to 3,000 x g)
- Vortex for sample preparation
- Laboratory Balance
- Foil to cover the microplate

PRECAUTIONS

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, all reagents should be treated as potentially infections. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. All materials derived from animal sources are bovine spongiform encephalopathy (BSE) negative. However, all materials should be kept from ruminating animals.
3. Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
4. The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
5. Avoid direct contact with skin.
6. This product is not for internal use.
7. Avoid eating, drinking, or smoking when using this product.
8. Do not pipette any reagents by mouth.
9. Reagents from this kit are lot-specific and must not be substituted.
10. Do not use reagents beyond the expiration date.
11. Variations to the test procedure are not recommended and may influence the test results.

STORAGE CONDITIONS

The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label.

SAMPLE HANDLING

Stool and saliva samples are appropriate for use in this assay.

Saliva

To avoid variation in sIgA content, always take saliva samples at the same time of the day. No food or liquid should be consumed within 30 min before sample collection. Collect saliva samples using salivettes and centrifuge at 3,000 rpm for 10 min. For analysis, the saliva supernatant is diluted 1:2000 in ELISA wash buffer.
10 µL saliva supernatant + 990 µL wash buffer = dilution I (1:100)
50 µL dilution I + 950 µL wash buffer = dilution II (1:20).
Final dilution: 1:2,000. Use 100 µL of the final dilution per well.

**Extraction of the Stool Sample:** Diluted extraction buffer is used as a sample extraction buffer. It is recommended to perform the following sample preparation:

1. The raw stool sample has to be thawed. For particularly heterogeneous samples it is recommended to perform a mechanical homogenization using an applicator, inoculation loop or similar device.
2. Fill the empty sample tube with 1.5 mL of ready-to-use extraction buffer before using it with the sample. Important: Allow the extraction buffer to reach room temperature. If using the EZ Extraction Device, the device is provided in a prefilled format and does not require any additional extraction buffer.
3. Unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off and leave 15 mg of sample to be diluted. Screw tightly to close the tube.
4. Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for app. 10 minutes improves the result.
5. Allow sample to stand for app. 10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
6. Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again. This will result in a dilution of 1:100 (Dilution I).

**Dilution of samples:** The supernatant of the sample preparation procedure (dilution I) is further diluted 1:125 in wash buffer.

\[
40 \mu L \text{ dilution I} + 960 \mu L \text{ wash buffer (mix well)} = \text{dilution II (1:25)}
\]

\[
200 \mu L \text{ dilution II} + 800 \mu L \text{ wash buffer (mix well)} = \text{dilution III (1:5)}
\]

Final dilution: 1:12,500. For analysis, pipet 100 µL of dilution III per well.

**Stability of stool samples**
The sample stability is as follows: Raw stool: 24 hours at 4°C, 8 weeks at -20°C
Stool extracts (1:100): 1 day at room temperature (15–30°C), 7 days at 2–8°C or 7 days at -20°C; maximum 2 freeze-thaw cycles.

**REAGENT PREPARATION**
All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.

**Controls (Levels 1 and 2)** are provided in a lyophilized form. Reconstitute in 500 µL of distilled water. Close the vial with the rubber stopper and cap, gently swirl the vial, and allow it to stand for 10 minutes prior to use. The contents of the vial should be in solution with no visible particulates. Reconstituted controls can be stored at 2–8 °C for 4 weeks.
**Standards** are provided in a lyophilized form. Reconstitute in 500 µL of distill water. Close the vial with the rubber stopper and cap, gently swirl the vial, and allow it to stand for 10 minutes prior to use. The contents of the vial should be in solution with no visible particulates. Reconstituted standards can be stored at 2–8 °C for 4 weeks.

**Wash Buffer Concentrate (10X)** must be diluted with distilled water 1:10 before use (100 mL WASHBUF + 900 mL distilled water) and mixed well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be dissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solutions. The WASHBUF is stable at 2–8 °C until the expiry date stated on the label. Wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8 °C for one month.

**Extraction Buffer Concentrate (2.5X)** must be diluted with distilled water 1:2.5 before use (100 mL Extraction Buffer + 150 mL distilled water), mix well. Crystals could occur due to high salt concentration in the stock solutions. Before dilution, the crystals must be dissolved at 37°C in a water bath. The Extraction Buffer is stable at 2–8 °C until the expiry date stated on the label. Prepared extraction buffer (1:2.5 diluted) can be stored in a closed flask at 2–8 °C for three months.

**Conjugate Concentrate** must be diluted 1:101 in wash buffer (100 µL conjugate concentrate + 10 mL wash buffer). The CONJ is stable at 2–8 °C until expiry date stated on the label. Conjugate (1:101 diluted CONJ) is not stable and cannot be stored.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2–8 °C.

**QUALITY CONTROL**

Control samples should be analyzed with each run. Results generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

**ASSAY PROCEDURE**

All reagents and microplate strips (while sealed in foil pouch) should be equilibrated to room temperature prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, controls, and samples should be run in duplicate.

For automated ELISA processors the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact ALPCO.

1. Bring all reagents and samples to room temperature (15–30°C) and mix well.
2. Wash the pre-coated microtiter plate 5x with 250 µL ELISA wash buffer before use. After the final washing step, the inverted microtiter plate should be tapped on absorbent paper.
3. Add 100 µL of STD / SAMPLE / CTRL into respective well. See **Reagent Preparation** for control reconstitution instructions. A suggested plate layout is included.
4. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker.
5. Aspirate the contents of each well. Wash each well 5x with 250 µL of wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
6. Add 100 µL CONJ (conjugate) into each well.
7. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C).
8. Aspirate the contents of each well. Wash each well 5x with 250 µL of wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
9. Add 100 µL of SUB (substrate) into each well.
10. Incubate for 10–20 minutes at room temperature (15–30°C) in the dark.*
11. Add 100 µL of STOP (stop solution) into each well, mix thoroughly.
12. Determine absorption immediately with an ELISA reader at 450nm against 620nm (or 690nm) as a reference. If no reference wavelength is available, read only at 450nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405nm against 620nm as a reference.

* The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

**CALCULATION OF RESULTS**

The following algorithms can be used alternatively to calculate the results. It is recommended to use the 4 parameter algorithm:

1. 4 parameter algorithm: It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.001).
2. Point-to-point calculation: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.
3. Spline algorithm: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

**Saliva**

For the calculation of the saliva values, the results from the microplate reader must be multiplied by the dilution factor of 2,000.

**Stool**

For the calculation of the stool values, the results from the microplate reader must be multiplied by the dilution factor of 12,500.

Samples with concentrations above the measurement range (see definition below) must be further diluted and re-assayed. Please consider this greater dilution when calculating the results. Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified. The upper limit of the measurement range can be calculated as: Highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:
LoB × sample dilution factor to be used

**PERFORMANCE CHARACTERISTICS**

**Analytical Sensitivity**

The Zero-standard was measured 20 times. The detection limit was set as \( B_0 + 2 \, SD \) and estimated to be 13.4ng/mL.

**Precision: Within run (intra-assay) variation**

The within run precision is expressed as the percentage coefficient of variation (CV %). This was determined based on the mean and standard deviation of 20 replicates of a sample run in a single assay. The table below shows the results of 2 samples.

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>77.7 ng/mL</td>
<td>92.5 ng/mL</td>
</tr>
<tr>
<td><strong>CV (%)</strong></td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

**Precision: Between run (inter-assay) variation**

The between run precision is expressed as the percentage coefficient of variation (CV %). This was determined based on the mean and standard deviation across 2 assays of 12 measurements of a single sample. The table below shows the results of 2 samples.

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>102.4 ng/mL</td>
<td>1277.4 ng/mL</td>
</tr>
<tr>
<td><strong>CV (%)</strong></td>
<td>8</td>
<td>7.4</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
Spike and Recovery

Two samples were spiked with three different pancreatic elastase concentrations and measured using this assay (n = 2).

<table>
<thead>
<tr>
<th>Unspiked Sample µg/mL</th>
<th>Spike µg/mL</th>
<th>Expected ng/mL</th>
<th>Measured ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>103.7</td>
<td>150</td>
<td>253.7</td>
</tr>
<tr>
<td></td>
<td>103.7</td>
<td>75</td>
<td>178.7</td>
</tr>
<tr>
<td></td>
<td>103.7</td>
<td>50</td>
<td>153.7</td>
</tr>
<tr>
<td></td>
<td>103.7</td>
<td>25</td>
<td>128.7</td>
</tr>
<tr>
<td>2</td>
<td>100.3</td>
<td>150</td>
<td>250.3</td>
</tr>
<tr>
<td></td>
<td>100.3</td>
<td>75</td>
<td>175.3</td>
</tr>
<tr>
<td></td>
<td>100.3</td>
<td>50</td>
<td>150.3</td>
</tr>
<tr>
<td></td>
<td>100.3</td>
<td>25</td>
<td>125.3</td>
</tr>
</tbody>
</table>

Dilutional Linearity

Three samples were diluted and analyzed. The results are shown in the table below (n=3).

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Expected ng/mL</th>
<th>Measured ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126.8</td>
<td>126.8</td>
</tr>
<tr>
<td>1:2</td>
<td>63.4</td>
<td>65.5</td>
</tr>
<tr>
<td>1:4</td>
<td>31.7</td>
<td>35.1</td>
</tr>
<tr>
<td>1:8</td>
<td>15.9</td>
<td>25.6</td>
</tr>
<tr>
<td>2</td>
<td>184.9</td>
<td>184.9</td>
</tr>
<tr>
<td>1:2</td>
<td>92.5</td>
<td>93.7</td>
</tr>
<tr>
<td>1:4</td>
<td>46.2</td>
<td>52.1</td>
</tr>
<tr>
<td>1:8</td>
<td>23.1</td>
<td>21.9</td>
</tr>
</tbody>
</table>
Specificity
No cross reactivity to other proteins in stool and saliva.

SHORT ASSAY PROTOCOL

- Wash Plate 5 times
- Add 100 µL standards, controls, and samples
- Incubate for 1 hr at RT
- Wash 5 times
- Add 100 µL Conjugate
- Incubate for 1 hr at RT
- Wash 5 times
- Add 100 µL Substrate
- Incubate for 10-20 min at RT in the dark
- Add 100 µL Stop Solution
- Read plate

Total Time = 2 hours 20 minutes
SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running standards, controls, and up to 40 samples in duplicate.

<p>| | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Std 1</td>
<td>Std 1</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>17</td>
<td>17</td>
<td>25</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td>B</td>
<td>Std 2</td>
<td>Std 2</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>18</td>
<td>18</td>
<td>26</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>C</td>
<td>Std 3</td>
<td>Std 3</td>
<td>3</td>
<td>3</td>
<td>11</td>
<td>11</td>
<td>19</td>
<td>19</td>
<td>27</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>D</td>
<td>Std 4</td>
<td>Std 4</td>
<td>4</td>
<td>4</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>20</td>
<td>28</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>E</td>
<td>Std 5</td>
<td>Std 5</td>
<td>5</td>
<td>5</td>
<td>13</td>
<td>13</td>
<td>21</td>
<td>21</td>
<td>29</td>
<td>29</td>
<td>37</td>
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<tr>
<td>F</td>
<td>Zero</td>
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<td>14</td>
<td>22</td>
<td>22</td>
<td>30</td>
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<td>38</td>
</tr>
<tr>
<td>G</td>
<td>Ctrl 1</td>
<td>Ctrl 1</td>
<td>7</td>
<td>7</td>
<td>15</td>
<td>15</td>
<td>23</td>
<td>23</td>
<td>31</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>H</td>
<td>Ctrl 2</td>
<td>Ctrl 2</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>16</td>
<td>24</td>
<td>24</td>
<td>32</td>
<td>32</td>
<td>40</td>
</tr>
</tbody>
</table>

Std= Standard
Ctrl = Control
Numbered wells = Samples