Secretory IgA ELISA

Catalog Number SE120114
Storage Temperature 2–8 °C

Product Description
The Secretory IgA (sIgA) ELISA kit is intended for the quantitative measurement of sIgA in human stool and saliva. The Secretory IgA (sIgA) ELISA kit is a solid phase direct ELISA sandwich method. The standards, samples, and controls are added into designated wells, coated with anti-sIgA monoclonal antibody, along with the incubation buffer. After a simple washing step, an anti-sIgA enzyme conjugate reagent is added into each well. After the excess Enzyme Conjugate is washed out, a chromogenic Substrate (TMB) is added into each well. Upon the addition of the Substrate, the intensity of color developed is directly proportional to the concentration of sIgA in the samples. A standard curve is generated relating color intensity to the concentration of sIgA.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell plate coated with anti-sIgA monoclonal Ab</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>sIgA Standard: 7 vials (ready to use)</td>
<td>0.125 mL</td>
</tr>
<tr>
<td>Anti-sIgA Enzyme Conjugate: 1 vial (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>sIgA Bi-level Control: 2 vials (ready to use)</td>
<td>0.125 mL</td>
</tr>
<tr>
<td>Incubation Buffer: 1 bottle (Ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>sIgA Sample Diluent: 3 bottles</td>
<td>3 x 20 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>20X Wash concentrate: 1 bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sample Preparation
1. sIgA is extracted by the sample diluent out of the stool sample.
2. Saliva samples should be centrifuged at 3,000 rpm for ten minutes.
3. Specimens may be stored refrigerated at (2–8 °C) for 5 days. If storage time exceeds 5 days, store frozen at (−20 °C) for up to one month.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen samples should be completely thawed and mixed well.

Stool Samples
Dilute extracted stool samples 1:500 in Sample Diluent.

Saliva Sample
Dilute the supernatant saliva samples 1:500 in Sample Diluent.

Wash Concentrate
Prepare 1x Wash Buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).
Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and samples be run in duplicate.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18–26 °C).

1. Format the microplate wells for each standard, control, and sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal, and store at 2–8 °C.
2. Pipette 10 µL of the standards, controls, and diluted samples into assigned wells.
3. Add 100 µL of incubation buffer into each well.
4. Cover plate and incubate for 60 minutes at room temperature, with shaking (600 rpm).
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer (see Preparation Instructions). Blot on absorbent paper towels.
6. Add 100 µL of anti-sIgA enzyme conjugate reagent into all wells.
7. Cover plate and incubate for 30 minutes at room temperature (18–26 °C), with shaking (600 rpm).
8. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash Buffer. Blot on absorbent paper towels.
9. Add 100 µL of TMB Substrate solution to each well.
10. Cover and incubate the plate for 15 minutes at room temperature (18–26 °C).
11. Add 50 µL of Stop Solution to each well and gently mix for 10 seconds.
12. Read the absorbance on ELISA Reader of each well at 450 nm within 15 minutes after adding the Stop Solution.

Results
Calculations
The standard curve is constructed as follows:
1. Check sIgA standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for sIgA standards (vertical axis) versus sIgA standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

<table>
<thead>
<tr>
<th></th>
<th>OD 450 nm</th>
<th>Concentration (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>Std 2</td>
<td>0.2</td>
<td>6.25</td>
</tr>
<tr>
<td>Std 3</td>
<td>0.38</td>
<td>12.5</td>
</tr>
<tr>
<td>Std 4</td>
<td>0.71</td>
<td>25</td>
</tr>
<tr>
<td>Std 5</td>
<td>1.21</td>
<td>50</td>
</tr>
<tr>
<td>Std 6</td>
<td>1.81</td>
<td>100</td>
</tr>
<tr>
<td>Std 7</td>
<td>2.53</td>
<td>200</td>
</tr>
</tbody>
</table>

Expected values
It is recommended that each laboratory establish their own range of expected values for the population being tested.

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient history, physical findings, and other diagnostic procedures.

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