**Example of a Standard Curve**

| | OD 450 nm | Conc. µg/dL |
|---|---|---|
| **Std 1** | 0.02 | 0 |
| **Std 2** | 0.2 | 6.25 |
| **Std 3** | 0.38 | 12.5 |
| **Std 4** | 0.71 | 25 |
| **Std 5** | 1.21 | 50 |
| **Std 6** | 1.81 | 100 |
| **Std 7** | 2.53 | 200 |

**LIMITATIONS OF THE TEST**

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

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Calbiotech Inc.,

CALBIOTECH
A life science company

# Secretory IgA ELISA

Catalog No. SC221A (96 tests)

**INTENDED USE**

**For Research Use Only. Not for use in diagnostic procedures.**

**PRINCIPLE OF THE TEST**

The Calbiotech Inc. Secretory IgA (sIgA) 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.

| MATERIALS PROVIDED | | 96 tests |
|---|---|---|
| 1. | Microwell plate coated with anti-sIgA monoclonal Ab | 12x8x1 |
| 2. | sIgA Standard: 7 vials (ready to use) | 0.125ml |
| 3. | Anti-sIgA Enzyme Conjugate: 1 vial (ready to use) | 12ml |
| 2. | sIgA Bi-level Control: 2 vials (ready to use) | 0.125ml |
| 5. | Incubation Buffer: 1 Bottle (Ready to use) | 12ml |
| 6. | sIgA Sample Diluent: 3 Bottles | 3 x 20ml |
| 7. | TMB Substrate: 1 Bottle (ready to use) | 12ml |
| 8. | Stop Solution: 1 Bottle (ready to use) | 12ml |
| 9. | 20X Wash concentrate: 1 bottle | 25ml |

**MATERIALS NOT PROVIDED**

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

## STORAGE AND STABILITY

1. Store the kit at 2 – 8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

## WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory Use.
3. Potential biohazardous materials:
   The standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. 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.

## SPECIMEN COLLECTION AND PREPARATION

1. sIgA is extracted by the sample diluent out of the stool sample.
2. Saliva samples should be centrifuged at 3000rpm for ten minutes.
3. Typically, 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.

## REAGENT PREPARATION

1. **Stool Samples:** Dilute extracted stool samples 1: 500 in sample diluent.
2. **Saliva Sample:** Dilute the supernatant saliva samples 1: 500 in sample diluents.
3. **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 (20-25°C).

## ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C).

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

## CALCULATION OF RESULTS

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 you check the value on every kit. See example of the standard attached**.**
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.