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INTENDED USE

The Calbiotech Inc. Secretory IgA (sIgA) ELISA Kit is intended for the quantitative measurement of sIgA in human stool and saliva. For Research Use Only. For professional use only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

Secretory IgA is the predominant antibody in mucosal secretions, playing a critical role in immune exclusion at mucosal surfaces. Research includes mucosal immunology, host–microbe interactions, vaccine efficacy in mucosal immunity, and polymeric immunoglobulin receptor biology.

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-slgA monoclonal Ab	12x8x1
2.	slgA Standard: 7 Vials (ready to use)	0.125 mL
3.	Anti-slgA Enzyme Conjugate: 1 Vial (ready to use)	12 mL
4.	slgA Bi-level Control: 2 Vials (ready to use)	0.125 mL
5.	Incubation Buffer: 1 Bottle (Ready to use)	12 mL
6.	slgA Sample Diluent: 3 Bottles	3 x 20 mL
7.	TMB Substrate: 1 Bottle (ready to use)	12 mL
8.	Stop Solution: 1 Bottle (ready to use)	12 mL
9.	20X Wash Concentrate: 1 Bottle	25 mL

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

- 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.



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WARNINGS AND PRECAUTIONS

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.

- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- 4. It is recommended that standards, control and serum samples be run in duplicate.
- 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. slgA is extracted by the sample diluent out of the stool sample.
- 2. Saliva samples should be centrifuged at 3000rpm for ten minutes.
- 3. This assay is designed for use in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze—thaw cycles.

REAGENT PREPARATION

- Stool Samples: Dilute extracted stool samples 1: 500 in sample diluent.
- 2. Saliva Sample: Dilute the supernatant saliva samples 1: 500 in sample diluents.
- 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).

- 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-slgA 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 slgA 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.
- To construct the standard curve, plot the absorbance for slgA standards (vertical axis) versus slgA standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

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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

	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.