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Product Information

Secretory IgA ELISA kit

Catalog Number: EA100882
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The OriGene Secretory IgA (sIgA) ELISA Kit is intended for the quantitative measurement of sIgA in human stool and saliva.

Principle of the Test

The 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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell plate coated with anti-sIgA monoclonal Ab	12x8x1
2. sIgA Standard: 7 vials (Ready to use)	125 µl
3. Anti-sIgA Enzyme Conjugate: 1 vial (Ready to use)	12 ml
2. sIgA Bi-level Control: 2 vials (Ready to use)	125 µl
5. Incubation Buffer: 1 Bottle (Ready to use)	12 ml
6. sIgA 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 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 450nm
5. Absorbance paper or paper towel

6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. sIgA is extracted by the sample diluent out of the stool sample.
2. Saliva samples should be centrifuged at 3000 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 sera 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 Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- 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 serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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 10 µl of the standards, controls and diluted samples into the assigned well.
3. Add 100 µl of incubation buffer into each well.
4. Cover plate and incubate for 60minutes at room temperature, with shaking (600 rpm)
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 100 µl of anti-sIgA enzyme conjugate reagent into all wells.
7. Cover plate and incubate for 30minutes, at room temperature, with shaking (600 rpm)
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 100 µl of TMB substrate solution to all wells
10. Cover and incubate the plate for 15 minutes at room temperature.
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 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.

Example of a Standard Curve

	Conc. (µg/dL)	OD 450 nm
Std 1	0	0.02
Std 2	6.25	0.2
Std 3	12.5	0.38
Std 4	25	0.71
Std 5	50	1.21
Std 6	100	1.81

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