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Product Information

Alpha 1-Antichymotrypsin ELISA kit

Catalog Number: EA100879
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Alpha 1-Antichymotrypsin (ACT) ELISA Kit is intended for the quantitative measurement of alpha 1-antichymotrypsin in human stool.

Background

The Alpha 1-Antichymotrypsin (ACT) kit is a solid phase direct ELISA sandwich method. The standards, samples and controls are added into designated wells, coated with anti-ACT polyclonal antibody, along with the incubation buffer. After a simple washing step, an anti-ACT enzyme conjugate reagent is added into each well. After the excess enzyme conjugate is washed out, the substrate is added into each well. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of ACT in the samples. A standard curve is generated relating color intensity to the concentration of ACT.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell plate coated with anti-ACT Polyclonal Ab	12x8x1
2. Alpha 1-Antichymotrypsin Standard: 8 vials (ready to use)	0.2 ml
3. Alpha 1-Antichymotrypsin Controls: 2 vials	0.2 ml
4. Anti-ACT Enzyme Conjugate: 1 vial (ready to use)	12 ml
5. Incubation Buffer: 1 bottle (ready to use)	12 ml
6. Sample Diluent: 3 bottles	3x22 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. Alpha 1-Antichymotrypsin is extracted by the sample diluent out of the stool sample.
2. 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.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen samples should be completely thawed and mixed well.

Reagent Preparation
1. **Samples**: Dilute stool samples 1: 1000 in sample diluent.
2. **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). 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 all wells.
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. Blot on absorbent paper towels.
6. Add 100 µl of anti-alpha 1-antichymotrypsin enzyme conjugate solution into all wells.
7. Incubate the plate for 30 minutes, at room temperature, with shaking (600 rpm).
8. Remove liquid from all wells. Wash wells three times with 300 of 1X wash buffer. 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 ACT 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 ACT standards (vertical axis) versus ACT 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.
4.

Example of a Standard Curve

	OD 450nm	Conc. (ng/Ml)
Std 1	0.04	0
Std 2	0.10	6.25
Std 3	0.17	12.5
Std 4	0.31	25
Std 5	0.55	50
Std 6	0.98	100
Std 7	1.61	200
Std 8	2.58	400

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