Human Alpha 1 Antichymotrypsin OneStep ELISA Kit

Catalog number: EK7018

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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Human Alpha 1 Antichymotrypsin OneStep ELISA Kit

Catalog Number: EK7018

Size: 96wells/kit

Sample Type: Serum and Plasma

Sensitivity: 6.25 ng/ml

Assay Range: 6.25-400 ng/mL

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

The Bosterbio OneStep ELISA kit is a solid phase sandwich ELISA kit. Instead of incubating with standards, samples and controls and with enzyme conjugated detection antibody separately, the OneStep ELISA kit allows the user to add standards, samples and controls to wells without incubation, and an enzyme conjugate reagent is added into each well immediately. Then incubate standards, samples, controls and enzyme conjugate reagent together. After the excess enzyme conjugate is washed out, the substrate is added into each well. The enzyme catalyzes the substrate yielding a blue color (Amax = 370nm and 652nm) that changes to yellow (Amax = 450nm) upon addition of a sulfuric or phosphoric acid stop solution. The intensity of color developed is directly proportional to the concentration of target protein in the samples. A standard curve is generated relating color intensity to the concentration of target protein.

Kit Components

Description	Quantity
Microwell plate coated with anti-ACT Polyclonal Ab	12x8x1
Alpha 1-Antichymotrypsin Standard: 8 vials (ready to use)	0.2ml
Alpha 1-Antichymotrypsin Controls : 2 vials	0.2ml
Anti-ACT Enzyme Conjugate: 1 vial (ready to use)	12ml
Incubation Buffer: 1 bottle (ready to use)	12ml
Sample Diluent: 3 bottles	3x22ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
20X Wash concentrate: 1 bottle	25ml

Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

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5. Absorbance paper or paper towel

6. Graph paper

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

SPECIMEN COLLECTION AND HANDLING

1. Alpha 1-Antichymotrypsin is extracted by the sample diluent out of the stool sample.
2. 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.
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).
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 10ul of the standards, controls and diluted samples into the assigned well.
3. Add 100ul of incubation buffer into all wells.
4. Cover plate and incubate for 60minutes, at room temperature, with shaking (600rpm)
5. Remove liquid from all wells. Wash wells three times with 300ul of 1X wash buffer The standard curve is constructed as follows:
6. Add 100ul of anti-alpha 1-antichymotrypsin enzyme conjugate solution into all wells.

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7. Incubate the plate for 30 minutes, at room temperature, with shaking (600rpm).
8. Remove liquid from all wells. Wash wells three times with 300ul of 1X wash buffer The standard curve is constructed as follows:
9. Add 100ul of TMB substrate solution to all wells
10. Cover and incubate the plate for 15 minutes at room temperature.
11. Add 50ul 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 Lysozyme standards (vertical axis) 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.

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