3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Alpha 1-Antichymotrpsin (ACT) ELISA Kit

Catalog Number **SE120143**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Alpha 1-Antichymotrypsin (ACT) ELISA Kit uses 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.

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

Components

Materials provided	96 Tests
Microwells coated with anti-ACT polyclonal Ab	12 x 8 x 1
Alpha 1-antichymotrypsin Standard: 8 vials	0.2 mL
Alpha 1-Antichymotrypsin Controls: 2 vials	0.2 mL
Anti-ACT Enzyme Conjugate: 1 vial	12 mL
Incubation Buffer: 1 bottle	12 mL
Sample Diluent: 3 bottles	3 x 22 mL
TMB Substrate 1 bottle	12 mL
Stop Solution 1 bottle	12 mL
20x Wash Concentrate: 1 bottle	25 mL

Reagents 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 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Alpha 1-Antichymotrypsin is extracted from the stool sample with the sample diluent. Dilute stool samples 1:1000 in sample diluent.
   Note: Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

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

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 (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

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.

Before proceeding with the assay, bring all reagents, standards, and controls to room temperature (18–26 °C).

1. Format the microplate wells for each standard, control, and samples 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 wells.
3. Add 100 µL of Incubation Buffer into all wells.
4. Cover plate and incubate for 60 minutes, at room temperature, with shaking (600 rpm).
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer (see Reagent Preparation). 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 µL of 1x wash buffer (see Reagent Preparation). 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 450 nm within 15 minutes after adding the Stop Solution.

Results

Calculations

The standard curve is constructed as follows:

1. Check the ACT standard value on each standard vial. This value might vary from lot to lot.
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.

Figure 1.
Sample Data for a Standard Curve

	OD 450 nm	Concentration (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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