Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

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Alpha 1-Antichymotrypsin ELISA Kit

(Catalog # E4702-100 ; 96 assays ; Storage at 2-8°C)

I. Introduction:

ACT functions as a specific inhibitor of chymotrypsin-like serine proteases. BioVision 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.

II. Sensitivity:

- 6.25 ng/ml

III. Sample Type:

- Stool

IV. Kit Contents:

Components	E4702-100	Part Number
Microwell plate coated with anti-ACT Polyclonal Ab	12 x 8	E4702-100-1
Alpha 1-Antichymotrypsin Standard: 8 vials (ready to use)	0.2ml	E4702-100-2
Alpha 1-Antichymotrypsin Controls : 2 vials	0.2ml	E4702-100-3
Anti-ACT Enzyme Conjugate: 1 vial (ready to use)	12 ml	E4702-100-4
Incubation Buffer: 1 bottle (ready to use)	12 ml	E4702-100-5
Sample Diluent: 3 bottles	3 x 22ml	E4702-100-6
TMB Substrate: 1 bottle (ready to use)	12 ml	E4702-100-7
Stop Solution: 1 bottle (ready to use)	12 ml	E4702-100-8
20X Wash concentrate: 1 bottle	25 ml	E4702-100-9

V. User Supplied Reagents and Equipment:

- deionized water, plate reader capable of reading absorbance at 450 nm

VI. Storage Conditions and Reagent Preparation:

Store the kit at 2°C – 8°C.

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).

VII. Assay Protocol:

1. Samples preperation: Dilute stool samples 1: 1000 in sample diluent. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

2. Assay procedure:

i. Format the microplate wells for each standard, control and patient specimen to be assayed in duplicate.
ii. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
iii. Pipette 10µl of the standards, controls and diluted samples into the assigned well.
iv. Add 100µl of incubation buffer into all wells.
v. Cover plate and incubate for 60minutes, at room temperature, with shaking (600 rpm)
vi. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
vii. Add 100 µl of anti-alpha 1-antichymotrypsin enzyme conjugate solution into all wells.
viii. Incubate the plate for 30 minutes, at room temperature, with shaking (600 rpm).
ix. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
x. Add 100µl of TMB substrate solution to all wells
xi. Cover and incubate the plate for 15 minutes at room temperature.
xii. Add 50µl of stop solution to each well and gently mix for 10 seconds.
xiii. Read the absorbance on ELISA Reader of each well at 450 nm within 15 minutes after adding the stop solution.

3. Standard Curve: 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. 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.

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

4. **Calculation:** Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

VIII. Related Products:

Alpha 1 Antichymotrypsin, Human Plasma (7293)

FOR RESEARCH USE ONLY! Not to be used on humans.