Human Elastase ELISA Kit

Product Code: 248932

Product Introduction

This kit is used to determine the content of elastase in samples.

This assay uses a double-antibody sandwich method to determine the level of human elastase in specimens. Purified human elastase antibody is coated onto the microplate wells to form a solid-phase antibody. Elastase in the sample binds to the coated antibody and then to the HRP-labeled elastase antibody, forming an antibody-antigen-enzyme-labeled antibody complex.

After thorough washing, add TMB substrate for color development. Under the catalysis of HRP, TMB turns blue, and after acid is added it becomes yellow. The color intensity is positively correlated with the elastase concentration in the sample. Measure the absorbance at 450 nm with a microplate reader and calculate the concentration of human elastase from the standard curve.

Product Packing List

Package size: 48T

Code	Item	Specification
248932.1	20-fold concentrated wash solution	20 mL x 1 bottle
248932.2	Enzyme conjugate reagent	3 mL x 1 bottle
248932.3	Sample Diluent	3 mL x 1 bottle
248932.4	Chromogenic Reagent A Solution	3 mL x 1 bottle
248932.5	Chromogenic Reagent B Solution	3 mL x 1 bottle
248932.6	Stop Solution	3 mL x 1 bottle
248932.7	Standard (800 ng/mL)	0.5 mL x 1 bottle
248932.8	Standard Diluent	1.5 mL x 1 bottle
248932.9	Precoated Microplate	12 wells x 4 strips
248932.10	Plate Sealer	2 sheets
248932.11	Sealed Bag	1 pc
248932.m	Manual	1 copy

Quality Standards and Safety Instructions

Raw Material and Packaging Name	Quality Standard	Primary Toxicity
20 x Concentrated Wash Solution	--	--
Enzyme-Labeled Reagent	--	--
Enzyme-Labeled Coated Plate	--	--
Sample Diluent	--	--
Chromogen A Solution	--	--
Chromogen B Solution	--	--
Stop Solution	--	--
Standard (800 ng/mL)	--	--
Standard Diluent	--	--
Plate Sealing Film	--	--
Sealed Bag	--	--

Transport and Storage Conditions

Transport: Transport with ice packs.

Storage: Store at 2-8 C. Valid for 180 days.

Instructions for Use

1. Procedure

1.1 Standard Dilution

This kit provides one vial of undiluted standard. Dilute it in small test tubes according to the following table.

Standard	Target Concentration	Preparation
No. 5	400 ng/mL	Add 150 uL of the undiluted standard to 150 uL of standard diluent.
No. 4	200 ng/mL	Add 150 uL of No. 5 standard to 150 uL of standard diluent.
No. 3	100 ng/mL	Add 150 uL of No. 4 standard to 150 uL of standard diluent.
No. 2	50 ng/mL	Add 150 uL of No. 3 standard to 150 uL of standard diluent.
No. 1	25 ng/mL	Add 150 uL of No. 2 standard to 150 uL of standard diluent.

1.2 Assay Steps

1. Set up blank wells, standard wells, and sample wells. The blank control wells do not receive sample or enzyme conjugate reagent; all remaining steps are the same.
2. Add 50 uL of standard to each standard well.
3. Add 40 uL of sample diluent to each sample well, then add 10 uL of sample. The final sample dilution is 5 times.
4. When adding sample, place it at the bottom of the well, avoid touching the wall, and gently shake to mix evenly.
5. Seal the plate and incubate at 37 C for 30 minutes.
6. Before use, dilute the 20 x concentrated wash solution 20-fold with distilled water.
7. Remove the sealing film carefully, discard the liquid, shake dry, and fill each well with wash solution. Let stand for 30 seconds, discard, and repeat 5 times. Pat dry.
8. Add 50 uL of enzyme conjugate to each well except the blank well.
9. Seal and incubate again at 37 C for 30 minutes.
10. Wash again according to the wash procedure above.
11. Add 50 uL of Chromogenic Reagent A, then 50 uL of Chromogenic Reagent B. Gently shake to mix.
12. Develop color at 37 C for 15 minutes protected from light.
13. Add 50 uL of stop solution to each well to terminate the reaction. The blue color immediately turns yellow.
14. Zero the instrument with the blank well, then read the absorbance of each well at 450 nm. Perform the measurement within 15 minutes after adding the stop solution.

> The detailed procedure above specifies color development for 15 minutes. A later overview line in the source text states 10 minutes.

2. Calculation of Results

Use the concentration of the standards as the horizontal coordinate and the OD value as the vertical coordinate to draw a standard curve on graph paper. Find the corresponding sample concentration from the sample OD value, then multiply by the dilution factor.

Alternatively, use the standard concentrations and OD values to calculate a linear regression equation for the standard curve. Substitute the sample OD value into the equation to calculate the sample concentration, then multiply by the dilution factor to obtain the actual concentration.

3. Overview of the Test Procedure

1. Prepare reagents, samples, and standards.
2. Add the prepared samples and standards, then incubate at 37 C for 30 minutes.
3. Wash the plate 5 times, add enzyme conjugate, and incubate at 37 C for 30 minutes.
4. Wash the plate 5 times, add color developing solutions A and B, and develop color at 37 C for 10 minutes.
5. Add stop solution.
6. Read the OD value within 15 minutes.
7. Calculate the results.

Precautions

1. After removal from refrigerated storage, allow the kit to equilibrate at room temperature for 1 hour before use. If the enzyme-coated plate is not used up after opening, place the strips in a sealed bag for storage.
2. Concentrated wash solution may show crystal precipitation. It can be warmed in a water bath during dilution to aid dissolution. This does not affect washing results.
3. Use a pipettor for sample addition at each step and check its accuracy frequently to avoid test errors. A single round of sample addition should preferably be completed within 5 minutes. If many specimens are tested, a multichannel pipette is recommended.
4. Run a standard curve with each assay, preferably in duplicate. If the analyte level in the specimen is too high and the sample OD value is greater than the OD value of the first standard well, dilute the sample with sample diluent by n times before measurement. When calculating the result, multiply by the total dilution factor (x n x 5).
5. Plate sealing film is for single use only to avoid cross-contamination.
6. Store the substrate protected from light.
7. Follow the instructions strictly. Test results must be based on the microplate reader reading.
8. All samples, wash solution, and waste materials should be handled as infectious materials.
9. Do not mix components from different lot numbers.
10. Specimens should be extracted as soon as possible after collection. Perform extraction according to relevant literature and run the experiment as soon as possible after extraction. If the test cannot be performed immediately, the specimen may be stored at -20 C. Avoid repeated freeze-thaw cycles. Samples containing NaN3 cannot be tested because NaN3 inhibits the activity of horseradish peroxidase (HRP).
11. Detection range: 15 ng/mL-450 ng/mL.

Visual Reference