KAMIYA BIOMEDICAL COMPANY

Human Neutrophil Elastase ELISA

For the quantitative determination of neutrophil elastase in human ascites fluid, serum and urine

Cat. No. KT-1884

For Research Use Only. Not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Human Neutrophil Elastase ELISA is an enzyme immunoassay for the quantitative determination of neutrophil elastase in human ascites fluid, serum and urine serum. For research use only. Not for use in diagnostic procedures.

INTRODUCTION

Neutrophil elastase (NE) is a serine protease that is secreted by neutrophils. Increased serum levels have been associated with colorectal cancer. Increased sputum levels have been found in COPD subjects with bacterial infection and in subjects with bronchiectasis.

PRINCIPLE

The NE ELISA uses affinity purified NE antibodies for solid phase immobilization (microtiter wells) and horseradish peroxidase (HRP) conjugated NE antibody for detection. Calibrators and diluted samples are incubated in the microtiter wells for one hour. The wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. NE molecules are thereby sandwiched between the immobilization and detection antibodies. The wells are washed to remove unbound HRP-conjugate, and TMB reagent is added and incubated for 20 minutes. A blue color develops if NE is present. The assay is stopped by the addition of Stop Solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of NE is derived from a calibration curve.

COMPONENTS

- Anti-NE coated 96-well plate (12 x 8 wells)
- Anti-NE HRP conjugate, 11 mL
- NE stock (lyophilized), 2 vials
- Diluent, 50 mL
- 20X Wash Solution, 50 mL
- TMB, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes and tips
- Distilled or de-ionized water
- Polypropylene or microcentrifuge tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker (25℃/150 rpm)
- Plate washer
- Plate reader at capable of measuring at 450 nm
- Graphing software

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

CALIBRATOR PREPARATION

The NE stock is comprised of pure human NE lyophilized in a stabilizing carrier protein matrix.

1. Label 5 polypropylene tubes as 5, 2.5, 1.25, 0.625 and 3.13 ng/mL and dispense 250 µL of diluent into each tube.
2. Reconstitute the lyophilized NE stock as directed on the vial label. Mix gently. This provides the 10 ng/mL calibrator.
3. Pipette 250 µL of the 10 ng/mL NE calibrator into the tube labeled 5 ng/mL and mix. This provides the 5 ng/mL NE calibrator.
4. Similarly prepare the remaining calibrators by two-fold serial dilution.

SAMPLE PREPARATION

We tested ascites fluid samples from cancer subjects and found NE levels ranging from undetectable to >1,300 ng/mL. Because of the wide range of values, it is not possible to suggest a single dilution that is appropriate for all samples. However, ascites fluid, serum and urine should be diluted at least 20-fold in order to eliminate matrix effects. We suggest that samples be tested at an initial dilution of 100-fold. Plasma has not been tested in the assay.

GENERAL INSTRUCTIONS

1. This kit is for research purposes only. Under no circumstances should it be used for diagnostic purposes.
2. Please take the time to completely read all instructions before starting your assay. Contact us if you need clarification.
3. All reagents should be allowed to reach room temperature (25°C) before use.
4. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and adherence to good laboratory practice.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µL/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash and blot the plate as described in 4 and 5.
9. Dispense 100 µL of TMB into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm with a plate reader within 5 minutes.

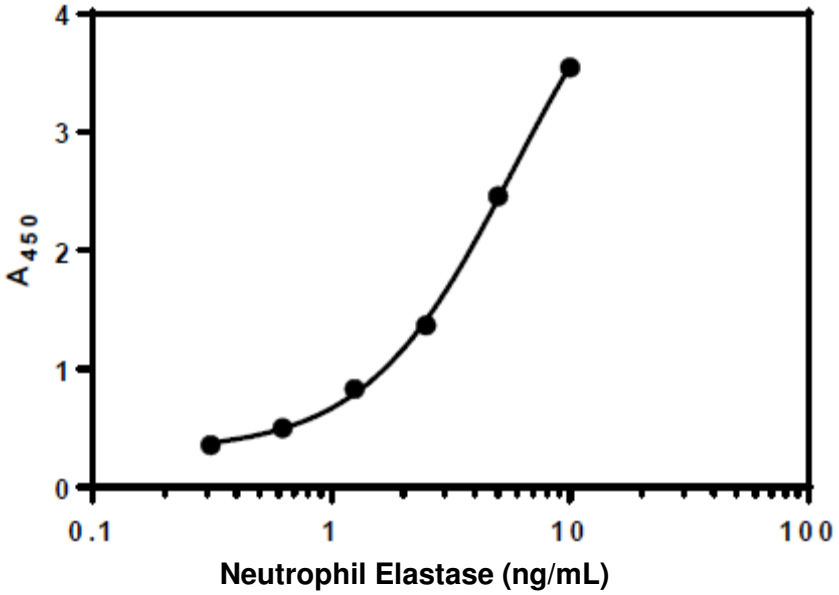
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration curve (remember to derive the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm on the Y-axis against NE concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

NE (ng/mL)	A_{450}
10	3.545
5	2.460
2.5	1.367
1.25	0.833
0.625	0.501
0.313	0.360



STORAGE

The NE stock should be stored at -20℃. The remainder of the kit should be stored at 4℃. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date provided that the components are stored appropriately.

FOR RESEARCH USE ONLY

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