- Following dilution, samples must be kept on ice until pipetted onto the microtitre plate.

## Precautions

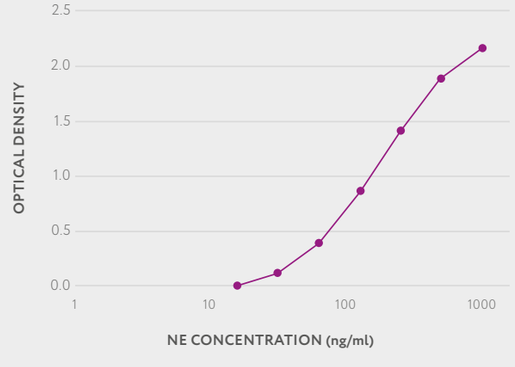
- NE-Tag is supplied in DMF which is a solvent classified as harmful and an irritant. Wear gloves and protective clothing when handling and avoid breathing fumes
- The NE standard and Stop Solution are acidic solutions
- The wash buffers and diluents within the kit contain preservatives which may cause an allergic skin reaction Do not breathe mist
- The substrate is classified as an irritant and can cause respiratory tract, eye and skin irritation
- Wear personal protective equipment including gloves, clothing, eye and face protection. Wash hands after handling reagents
- We recommend the use of external controls for internal quality control.

## Performance Characteristics

**Typical Results:**

This standard curve is provided for illustrative purposes only.

Each laboratory should produce its own standard curve for each set of samples analysed.




**Measuring Range:**

The measuring range of this immunoassay is 15.625 – 1000 ng/ml.

**Limit of Detection:**

The lowest concentration of NE giving an absorbance reading greater than 3 Standard Deviations (SD) above the mean zero (blank) reading (n=60) was determined to be 7.2 ng/ml.

**Precision:**

Intra-assay coefficient of variation (CV) was determined by measurement of replicates of spiked buffer and four CF Sol samples in one assay. Intra-Assay CV (within assay precision) = <10%.

Inter-assay CV was determined by measurement of spiked buffer and four CF Sol samples in 5-10 separate assays. Inter-Assay CV (between assay precision) = <10%.

**Analytical Recovery:**

BALf, sputum sol and serum free media (SFM) were diluted in NE Standard Diluent before being spiked with NE and analysed in the assay. Recovery was calculated from (measured/expected) expressed as a percentage. On average all samples were found to have a recovery range of 80-120%.

**Analytical Linearity:**

NE was measured in serial dilutions in BALf, sputum sol and SFM. Good linearity was noted for all samples examined, with linearity ranges of 100%-115%.

**Interference:**

Interference (inaccurate results) was observed when 0.1% (v/v) Dithiothreitol (DTT) is used during sample processing. It is recommended that samples containing DTT should be diluted by at least 100-fold for best results.

**Evidence of Deterioration:**

For questions or concerns regarding the performance or quality of products received, please contact ProAxsis directly.

## Symbols Used

| | | | |
|---|---|---|---|
| CE | EC Declaration of Conformity | | Manufactured By |
| RUO | Research Use Only | | Expiry Date |
| REF | Catalogue Number | | Storage Upper Limit of Temperature |
| LOT | Lot Number | | Storage Temperature Limitations |
| | Consult Instructions | | |

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# ProteaseTag® Active Neutrophil Elastase Immunoassay

Catalogue Number PA001US

## Instructions For Use

**Intended Use**

For the **in vitro** quantitative determination of active human neutrophil elastase (NE).

Read instructions for use in full before using this product.

CE RUO

## Assay Principle

The ProteaseTag® Active NE Immunoassay utilises ProteaseTag® technology to specifically detect and quantify active NE.

During a short incubation, the NE-Tag is coated onto the immunoassay plate before washing to remove excess. Standards and samples are added, and the NE-Tag will interact with the active site of NE during a second incubation step. Active NE present in the solutions will irreversibly bind to the NE-Tag while latent and inhibitor bound NE will be removed by washing. A horseradish peroxidase (HRP) conjugated anti-NE antibody is added to each test well and incubated. This detection antibody attaches to bound NE with unbound antibody subsequently removed by washing. A colour forming substrate containing tetramethylbenzidine (TMB) is added to each test well and reacts with HRP to generate a blue coloured product. This enzymatic reaction is subsequently stopped by the addition of acidic stop solution to each test well (a yellow solution is formed). The colour intensity (absorbance) is read at 450 nm using a plate reader.

## Materials Provided and Storage Conditions

Store the freezer box immediately upon receipt at -20°C.

Store the main kit box and components at 2 – 8°C.

Use all unopened reagents before their expiry date.

Immunoassay plates can be used on subsequent days. During use, cover unused wells with a plate seal. For storage, cover all used wells with a plate seal before placing inside foil pouch with the desiccant under recommended storage conditions.

Diluted Wash Buffer A and B remain stable for 24 hours. If not using all wells, dilute only the portion of NE Wash Buffer A and B Concentrate required. NE Wash Buffer A and B are stable for one month after opening if stored at 2 – 8°C within original container.

NE Reagent Diluent and NE Standard diluent are stable for one month after opening if stored at 2 – 8°C within original container.

Each vial of Human NE standard can only be used once.

NE Conjugate can be used twice when stored under recommended conditions.

Stop Solution, TMB Substrate and NE-Tag may be used multiple times when stored under recommended conditions.

## Kit Contents

| Freezer Box Components | |
|---|---|
| NE-Tag | 1 vial containing 4 µl (concentration 10 mM) of capture probe in N,N-dimethylformamide (DMF) |
| Human Neutrophil Elastase Standard | 2 vials containing 6 µl of native NE (concentration 100 µg/ml) in an acidic buffer |
| NE Conjugate | 1 vial containing 25 µl of anti-human neutrophil elastase antibody conjugated to horse radish peroxidase (HRP) in a stability solution |

| Main Kit Components | |
|---|---|
| Immunoassay Plate | 96 well streptavidin-coated microtitre plate (pre-blocked) |
| NE Wash Buffer A Concentrate | 24 ml of a 25-fold concentration of buffered surfactant with preservatives |
| NE Wash Buffer B Concentrate | 8 ml of a 25-fold concentration of buffer with preservatives |
| NE Standard Diluent | 15 ml of buffer with preservatives (ready to use) |
| NE Reagent Diluent | 2 x 13 ml of buffered surfactant with preservatives (ready to use) |
| TMB Substrate | 12 ml of tetramethylbenzidine (ready to use) |
| Stop Solution | 6 ml of 2 N sulphuric acid (ready to use) |
| Plate Sealers | 4 adhesive strips |

## Materials required but not supplied

- Deionised or distilled water
- Calibrated pipettes and corresponding pipette tips
- Tubes and tube racks
- Clean containers for diluted wash buffers
- Vortex
- Incubator (37°C)
- Calibrated microtitre plate reader capable of reading at 450 nm

## Reagent Preparation

With the exception of standards and samples, all reagents should be brought to room temperature before use. Standards and samples **must be kept on ice** at all times prior to use. Sufficient reagents are provided to allow for two standard curves.

- Wash Buffer A – Dilute 24 ml of the concentrate into 576 ml deionised water to prepare 600 ml of Wash Buffer A
- Wash Buffer B – Dilute 8 ml of the concentrate into 192 ml of deionised water to prepare 200 ml of Wash Buffer B
- NE Conjugate – Dilute 20 µl of NE Conjugate with 11980 µl of NE Reagent Diluent to prepare 12 ml of conjugate solution (600-fold dilution)
- NE-Tag – Dilute 2.4 µl of NE-Tag with 11997.6 µl of NE Reagent Diluent to prepare 12 ml of NE-Tag solution (5000-fold dilution)
- Standards –
  - Pipette 495 µl of NE Standard Diluent into the first micro-tube
  - Pipette 250 µl of NE Standard Diluent into 6 subsequent micro-tubes
  - Add 5 µl of the NE standard to the first tube. Carry out a dilution series by transferring 250 µl from one tube to the next. Mix each tube thoroughly before transferring to next tube. This will create a calibration curve with the following NE concentrations: 1000, 500, 250, 125, 62.5, 31.25 and 15.625 ng/ml
  - Use NE Standard Diluent as the zero (blank) standard
- Samples – dilute as appropriate using NE Standard Diluent.

## Assay Procedure

All samples and standards should be assayed in duplicate.

1. Prepare all reagents, standards and samples as directed previously. It is recommended that standards and samples are prepared immediately prior to adding to plate
2. Remove plate from the foil pouch
3. Aspirate and wash each well using 300 µl/well Wash Buffer A. Repeat the procedure twice for a total of 3 washes
4. Add 100 µl of diluted NE-Tag into each well. Cover with the plate sealer and incubate at 37°C for 30 minutes
5. Aspirate and wash each well using 300 µl/well **Wash Buffer B**. Repeat the procedure twice for a total of 3 washes
6. Add 100 µl of diluted standards, samples and blank into each well. Cover with the plate sealer and incubate at 37°C for 30 minutes
7. Aspirate and wash each well using 300 µl/well Wash Buffer A. Repeat the procedure twice for a total of 3 washes
8. Add 100 µl of diluted conjugate into each well. Cover with the plate sealer and incubate at 37°C for 60 minutes
9. Aspirate and wash each well using 300 µl/well Wash Buffer A. Repeat the procedure twice for a total of 3 washes
10. Add 100 µl of TMB Substrate solution into each well. Cover with the plate sealer and incubate at 37°C for 10 minutes. **Protect from light**
11. Add 50 µl of Stop Solution to each well. The colour within the wells should change from blue to yellow
12. Determine the optical density of each well within 15 minutes using a microplate reader set to 450nm.

## Calculation of Results

Average the duplicate readings for each standard, control and sample and subtract the zero blank from the final results. Plot a standard curve by generating a log x-Axis and fit the curve to spline or a 4 parameter logistic fit. If samples have been diluted, the concentration reported should be corrected for the dilution factor.

## Limitations of the Assay

- Store all components correctly
- Use all unopened reagents before their expiry date
- Do not mix or substitute reagents with those from other lots or sources
- All procedures should be carried out in accordance with the protocol
- If samples generate results higher than the highest standard, samples should be further diluted with NE Standard Diluent and repeated.

## Technical Hints

- This kit should only be use by qualified personnel
- Dispose of containers and unused contents in a safe way and in accordance with national and local regulations
- To avoid cross-contamination, change pipette tips between additions of each standard, sample and reagents. Also, use separate reservoirs for each reagent
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary
- A suggested dilution of sputum sol or bronchoalveolar lavage fluid (BALf) from individuals with cystic fibrosis is at least 100-fold with NE Standard Diluent. COPD samples have a suggested starting dilution of 10-fold