ProteaseTag® Active Neutrophil Elastase Immunoassay

Instructions for use

Version 1.0, created 09-2025

RUO

ProteaseTag® Active Neutrophil Elastase Immunoassay Test is a product for Research Use Only.

Revision History: PA001-RUO IFU

Revision	Date	Description
V-01	12 Sep 2025	First issue

1. Abbreviations and Definitions

Abbreviation	Description
IFU	Instructions for use
NE	Neutrophil elastase
HRP	horseradish peroxidase
TMB	tetramethylbenzidine
nm	nanometer
mL	milliliter
µl	microliter
µg	microgram
°C	degree Celsius
DMF	*N,N*-dimethylformamide

2. Statement of Intended Purpose

The ProteaseTag® Active neutrophil elastase (NE) Immunoassay is a single-use immunoassay based test for the quantitative determination of active human NE in sputum (sol) samples within a basic research or pharmaceutical research setting only. The product is intended for Research Use Only, it has no clinical applicability and should not be used for any clinical diagnostic use.

3. Principles of the Assay

The ProteaseTag® Active NE Immunoassay utilises ProteaseTag® technology to measure 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. 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.

4. Reagents and Equipment

a. Contents of the Main Kit

Main Kit Components	
Immunoassay Plate	96 well streptavidin-coated microtitre plate (pre-blocked)
NE Wash Buffer Concentrate	24 ml of a 25-fold concentration of buffered surfactant with preservatives
NE Reagent Diluent	4 x 12 ml of buffered surfactant with preservatives (ready to use)
TMB Substrate	10 ml of tetramethylbenzidine (ready to use)
Stop Solution	5 ml of 2 N sulphuric acid (ready to use)
Plate Sealers	4 adhesive strips

b. Contents of the Freezer Kit

Freezer Box Components	
NE-Tag	1 vial containing 2.4 µl (concentration 10 mM) of capture probe in *N,N*-dimethylformamide (DMF)
Human Neutrophil Elastase Standard	1 vial containing 2.5 µl of native NE (concentration 100 µg/ml) in an acidic buffer
NE Conjugate	1 vial containing 20 µl of anti-human neutrophil elastase antibody conjugated to horse radish peroxidase (HRP) in a stability solution

c. Materials Required but not Provided

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

d. Sample Material

The ProteaseTag® Active NE Immunoassay is intended for use with sputum sol samples only. The test requires a minimum of 100µl sputum sol per well, diluted in NE Reagent Diluent (Reference section 8 Performing the Test).

e. Storage of Reagents and Consumables

- 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.

5. Warnings and Precautions

- For Research Use Only. Not for use in Diagnostic procedures. The test is only intended for use by laboratory personnel appropriately trained in immunoassay techniques.
- NE-Tag is supplied in *N,N*-dimethylformamide (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 buffer and diluent 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.
- Refer to product SDS for further information on hazards and safety measures.
- 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 to prevent evaporation is necessary.
- Following dilution, samples **must be kept on ice** until pipetted onto the microtitre plate.
- 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.

6. Training

The test is only intended for use by laboratory personnel appropriately trained in immunoassay techniques and sputum sample handling. Please contact ProAxsis for information to support running the ProteaseTag® Active NE Immunoassay.

7. Waste Handling

Waste handling and disposal should adhere with the laws and regulations of the local and national authorities.

8. Performing the Test

a. 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. It is recommended to briefly centrifuge vials of NE-Tag, NE Standard, and NE Conjugate prior to use to collect contents at the bottom of the vial.

- **Wash Buffer** – Dilute 24 ml of the concentrate into 576 ml of deionised or distilled water to prepare 600 ml of Wash Buffer.
- **NE-Tag** - Dilute 2.4 µl of NE-Tag with 11,997.6 µl of NE Reagent Diluent to prepare 12 ml of NE-Tag solution (5000-fold dilution).

- **Standards** –
  - Pipette 997.5 µl of NE Reagent Diluent into the first micro-tube.
  - Pipette 250 µl of NE Reagent Diluent into 5 subsequent micro-tubes.
  - Add 2.5 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 six-point calibration curve with the following NE concentrations: 250, 125, 62.5, 31.25, 15.63 and 7.81 ng/ml.
  - Use NE Reagent Diluent as the zero (blank) standard.

- **Samples** – Dilute sputum sol samples using NE Reagent Diluent. A minimum dilution factor of x200 is recommended, however, this should be optimised by the end user.

- **NE Conjugate** – Dilute 20 µl of NE Conjugate with 11,980 µl of NE Reagent Diluent to prepare 12 ml of conjugate solution (600-fold dilution)

b. 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 placed on ice and 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. 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^{o}C for 30 minutes.
5. Aspirate and wash each well using 300 µl/well Wash Buffer. 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^{o}C for 30 minutes.
7. Aspirate and wash each well using 300 µl/well Wash Buffer. 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^{o}C for 60 minutes.
9. Aspirate and wash each well using 300 µl/well Wash Buffer. 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^{o}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.

c. 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 using a 4-parameter logistic fit. If samples have been diluted, the concentration reported should be corrected for the dilution factor.

9. Troubleshooting

- If samples generate results which fall outside the standard curve range, sample dilution should be adjusted and analysis repeated.

10. Results of the Assay

- The ProteaseTag® Active NE Immunoassay will provide a quantitative concentration measurement of active NE (ng/mL).

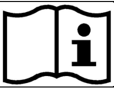
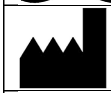
11. Limitations of the Procedure

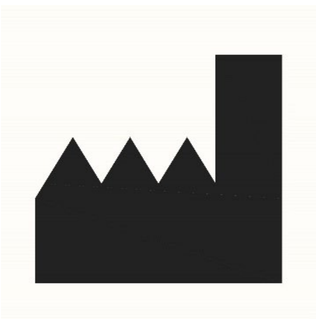
- For Research Use Only. Not for use in diagnostic procedures.
- The ProteaseTag® Active NE Immunoassay is intended for use with sputum sol samples only.
- The performance of the ProteaseTag® Active NE Immunoassay was validated using the procedures provided in this IFU only. Modification of these procedures may alter the performance of the test.

12. Quality Control

- We recommend the use of suitable controls (external/internal) for internal quality control.

13. Symbols

Symbol	Meaning
RUO	**Research Use Only**
REF	**Catalogue Number**
LOT	**Lot Number**
	**Consult Instructions**
	**Manufactured By**
	**Do Not Reuse, Intended For Single Use**
	**Expiry Date**
	**Storage Upper Limit of Temperature**
	**Storage Temperature Limitations**



ProAxsis Ltd
Building 75 Randox Science Park
30 Randalstown Road
Antrim, N. Ireland
BT41 4FL
Tel: +44
www.proaxsis.com

Product information: www.proaxsis.com
Orders: orders@proaxsis.com
Customer support: orders@proaxsis.com