EDN ELISA

For the quantitative determination of eosinophil-derived neurotoxin (EDN) in human stool and serum

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 31-EDNHU-E01

Size: 96 determinations

Version: 19.12.2025 / ALPCO 1.0

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INTENDED USE

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INTRODUCTION

Following activation, eosinophil granulocytes release the cationic glycoprotein EDN (eosinophil derived neurotoxin). This 18-21 kDa single stranded glycosylated protein is also known as EPX (eosinophil protein X). Together with ECP (eosinophil cationic protein), EDN belongs to the ribonuclease superfamily[1-3]. However, EDN has a 100-fold increased ribonuclease activity. It is neurotoxic, but not cytotoxic[4,5]. The activation of eosinophil granulocytes is important during inflammatory processes in allergic reactions. Thus, EDN is a marker for eosinophil activation and degranulation.

PRINCIPLE OF THE ASSAY

The EDN ELISA test determines human EDN according to the "sandwich" principle. EDN in samples, standards and controls binds to polyclonal antibodies which are coated to the microtiter plate. After a washing step, a peroxidase labeled polyclonal antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm in a microtiter plate reader. The EDN concentration can be calculated from the standard curve.

MATERIALS SUPPLIED

31-EDNHU-E01			
Component	**Quantity**	**Preparation**	**Storage**
Microtiter plate, coated	1 plate: 12 x 8-well strips	Ready-to-use	2 - 8˚C
Universal extraction buffer	150 mL	Ready-to-use	2 - 8˚C
ELISA Wash Buffer concentrate, 10X	100 mL	Dilute 1:10	2 - 8˚C
Standards*	7 vials, 2 mL each	Ready-to-use	2 - 8˚C
Control 1 and 2*	2 vials, 2.5 mL each	Ready-to-use	2 - 8˚C
Conjugate: peroxidase-labeled polyclonal antibody	15 mL	Ready-to-use	2 - 8˚C
TMB Substrate: tetramethylbenzidine	15 mL	Ready-to-use	2 - 8˚C
Stop solution	10 mL	Ready-to-use	2 - 8˚C
Plate Sealer	2	Ready-to-use	RT

*Please refer to the Certificate of Analysis enclosed with each kit for Standard and Control concentrations.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge, 3000 x g
- Plastic vials
- Stool sample extraction vials (80-EXDEV2-100) or serum collection tubes

- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate (substrate step)
- Distilled or deionized water
- ELISA reader with 450 nm filter (620 nm reference filter)
- Microtiter plate shaker (2 mm orbital shaker capable of 400 rpm)
- Vortex mixer
- Absorbent paper (lint-free)
- Timer

PRECAUTIONS

- This assay is for Research Use Only. Not for use in diagnostic procedures.
- Individual components from different batches and kits should not be interchanged. The expiry dates stated on the package must be observed.
- The kit reagents contain preservatives to prevent bacterial growth. avoid contact with the skin and/or mucous membranes.
- The kit contains EDN isolated from human urine from apparently healthy donors. During the purification process, all high-molecular components were separated. As a precaution, all kit reagents should always be treated as potentially infectious material in accordance with health care accident prevention regulations.
- The substrate TMB (tetramethylbenzidine) is toxic by ingestion and skin contact. In the event of contact with the skin, the affected area must be washed immediately with plenty of water and soap.
- Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. Work with protective gloves and goggles. In the event of contact, the burned area must be rinsed immediately and thoroughly with plenty of water. If necessary, a doctor should be consulted.
- Adherence to the prescribed protocol for performing the test is essential. ALPCO assumes no liability for any damage caused by unauthorized changes in the test procedure.
- Observe the guidelines for carrying out quality control in testing laboratories. Appropriate controls must be tested.
- Do not use reagents must after the expiration date.
- Wear disposable gloves when handling samples or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or apply makeup in areas where samples or kit reagents are handled.
- Samples may contain unknown interfering substances. This can lead to false high or false low results.

REAGENT PREPARATION

Microtiter plate: Assemble the required number of strips on the holder. Ensure the plate has reached 20-30°C before use. Strips which are not needed must be stored at 2-8°C in the pouch with desiccant. Do not dispose of the holder until all strips are used.

Wash buffer: Dilute the wash buffer concentrate 1:10 with distilled or deionized water (1 part buffer + 9 parts DI water). When storing the wash buffer concentrate at 2-8 °C, crystallization may occur. Before dilution, all crystals must be dissolved. The 1X working Wash Buffer is stable for 14 days at 2-8°C. IDilute only the amount of buffer which is needed to process the given samples.

All other test reagents are stable at 2-8 °C until the date of expiration stated on the label, unless otherwise specified.

SAMPLE PREPARATION

Stool Samples

Stool samples must be diluted 1:100 using Universal Extraction Buffer before testing in the EDN ELISA. Manual weighing or an approved stool extraction device (80-EXDEV2-100) may be used.

For manual weighing, mix **15 mg** stool with **1.5 ml** universal extraction buffer (or greater amount of stool diluted 1:100 with universal extraction buffer), then vortex until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge for 10 min at 3000 x g. 100 µl of the supernatant is used per well in the EDN ELISA.

Serum Samples

Serum samples must be centrifuged within 60 min (5 min, 3000 x g).

Place 50 µl of the supernatant into a new plastic vial. Dilute 1:5 with universal extraction buffer (50 µl sample + 200 µl universal extraction buffer) and mix thoroughly.

ASSAY PROCEDURE

All reagents and samples should be equilibrated to 20-30°C and mixed well before use.
The position of standards, controls, and samples are noted on a protocol sheet.

1. **Washing step**

   Pick out the pre-assembled microtiter plate with the needed number of strips and wash them 1x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the wash step.

2. **Sample incubation**

   Pipette **100 µl standards, controls, and samples** in duplicate in the microtiter plate.

   Cover and incubate for **60 min** at room temperature (20-30°C) with shaking (400 rpm, 2 mm orbit).

3. **Wash step**

Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

4. **Conjugate incubation**

   Pipette **100 µl conjugate** in each microwell. Cover and incubate for **60 min** at room temperature (20-30°C) with shaking (400 rpm, 2 mm orbit).

5. **Wash step**

   Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

6. **Substrate incubation**

   Pipette **100 µl substrate** in each microwell. Incubate for **10-15 min** at room temperature (20-30°C) with shaking (400 rpm, 2 mm orbit) in the dark.

7. **Stop the reaction**

   Pipette **50 µl stop solution** in each microwell. Mix well..

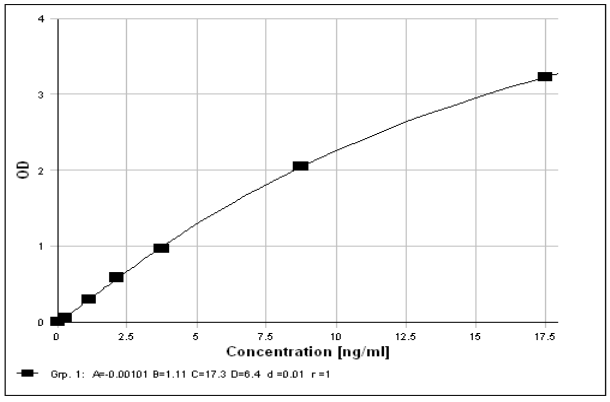
8. **Measurement**

   Read the absorbance at 450 nm. If available, use 620 nm as a reference wavelength. Reading should be done within **5 minutes** after stopping the reaction.

CALCULATION OF RESULTS

The use of the 4-parameter-Marquardt algorithm is recommended for calculation of results. For stool samples, the obtained EDN concentration is multiplied by the dilution factor of 100. For serum samples, the obtained EDN concentration is multiplied by the dilution factor of 5.

TYPICAL STANDARD CURVE



The curve at left is for demonstration purposes only. It must not be used for calculation of sample values.

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range of EDN in stool is 27.5 – 1,750 ng/ml (or µg/g). In serum, the measuring range is 1.375 – 87.5 ng/mL.

Sensitivity

Limit of Detection (LOD): 0.05 ng/mL

For the determination of the detection limit, 20 replicates of Standard 0 were measured. After addition of the 3-fold standard deviation to the mean value, the concentration was read from the standard curve.

Limit of Quantification (LOQ): 0.1 ng/mL

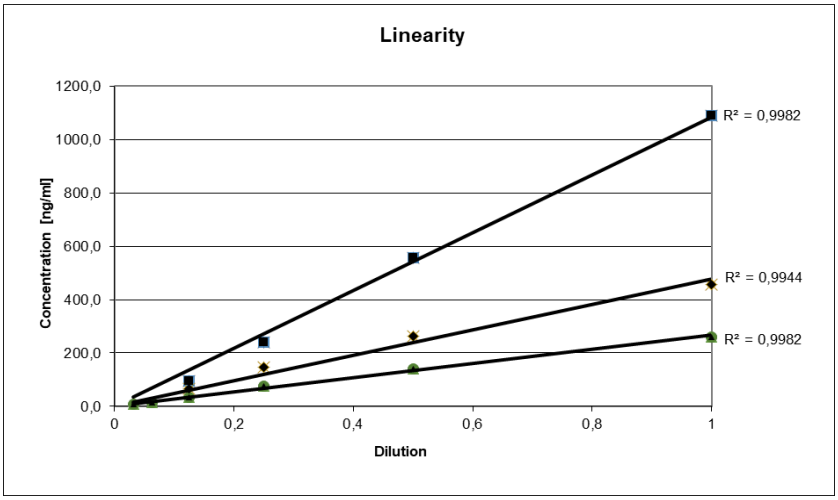
For the determination of the detection limit, 20 replicates of Standard 0 were measured. After addition of the 10-fold standard deviation to the mean value, the concentration was read from the standard curve.

Precision and reproducibility

Intra-Assay CV Stool:	<10 % (1672 ng/ml)	[n = 10]
	<10 % (730 ng/ml)	[n = 10]
	<10 % (197 ng/ml)	[n = 10]
Inter-Assay CV Stool:	<15 % (1178 ng/ml)	[n = 10]
	<10 % (446 ng/ml)	[n = 10]
	<10 % (218 ng/ml)	[n = 10]
Intra-Assay CV Serum:	<10 % (5.9 ng/ml)	[n = 10]
	<10 % (38.5 ng/ml)	[n = 10]
	<10 % (84.2 ng/ml)	[n = 10]
Inter-Assay Serum:	<10 % (5.8 ng/ml)	[n = 10]
	<10 % (38.8 ng/ml)	[n = 10]
	<10 % (83.8 ng/ml)	[n = 10]

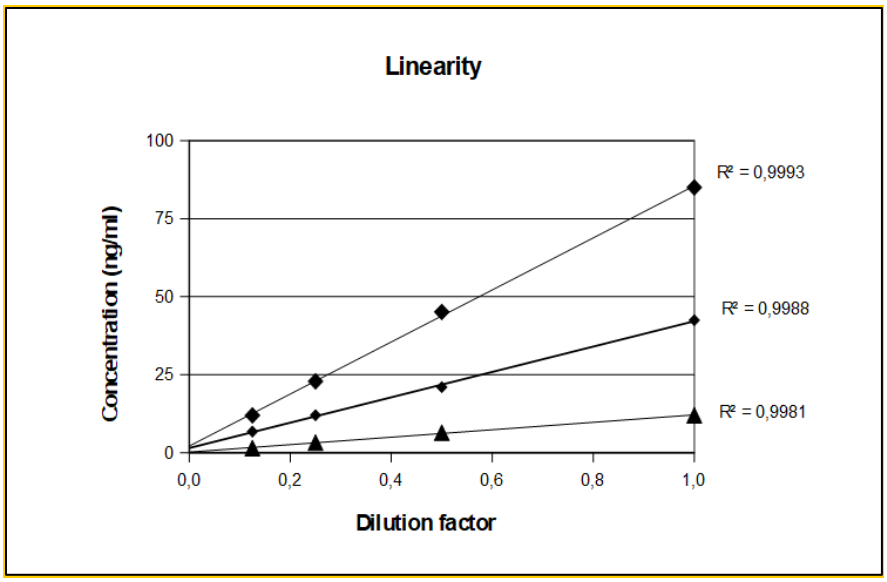
Linearity (Stool)

Sample	Dilution Factor	Expected (ng/mL)	Measured (ng/mL)	Recovery (%)
1	--	--	457	--
	1:2	229	263	115
	1:4	114	145	127
	1:8	57.2	65.4	114
2	--	--	1090	--
	1:2	545	555	102
	1:4	273	241	88.5
	1:10	109	94.9	87.0
3	--	--	261	--
	1:2	131	139	106
	1:4	65.3	76.8	118
	1:8	32.7	34.3	105
	1:16	16.3	13.8	84.5
	1:32	8.2	7.3	89.4



Linearity (Serum)

Sample	Dilution Factor	Expected (ng/mL)	Measured (ng/mL)	Recovery (%)
1	--	--	42.5	--
	1:2	21.2	21.0	98.9
	1:4	10.6	10.6	113.1
	1:8	5.3	5.3	127.2
2	--	--	85.0	--
	1:2	42.5	45.1	106.1
	1:4	22.9	22.9	107.5
	1:8	12.0	12.0	112.5
3	--	--	12.0	--
	1:2	6.0	6.0	106.7
	1:4	3.0	3.0	108.0
	1:8	1.5	1.5	98.7



Spike and Recovery (Stool)

Sample	Endogenous (ng/mL)	Added	Expected (ng/mL)	Measured (ng/mL)	Recovery (%)
1	2.5	4.2 12.5 25.0	6.6 15.0 27.5	6.9 15.3 30.6	105 102 111
2	5.2	4.2 12.5 25.0	9.4 17.7 30.2	9.0 17.9 26.8	95.4 101 88.7

Spike and Recovery (Serum)

Sample	Endogenous (ng/mL)	Added	Expected (ng/mL)	Measured (ng/mL)	Recovery (%)
1	45.3	17.5 35.0 52.5	62.8 80.3 98.0	59.0 73.0 83.5	93.9 90.9 85.4
2	29.1	17.5 35.0 52.5	46.6 64.1 81.6	42.2 60.0 74.0	90.7 93.7 90.7

Limitations of the Method

Samples with EDN concentrations above the standard curve should be diluted with universal extraction buffer and measured again.

Disposal

The substrate must be disposed of as non-halogenated solvent. The stop solution can be neutralized with NaOH, and if the pH value is neutral it can be disposed of as salt solution (**important:** this reaction produces heat and should be handled carefully). Please refer to local and national guidelines.

REFERENCES

1. Durack D.T., et al., Proc. Natl. Acad. Sci. USA 78, 5165 (1981).
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3. Slifman N.R., et al., J. Immunol.143, 2317 (1989).
4. Gullberg U., et al., Biochem. Biophis. Res. Commun. 139, 1239 (1986).
5. Slifman N.R., et al., J. Immunol. 137, 2913 (1986).