



EAGLE
BIOSCIENCES

Eosinophil Derived Neurotoxin (EDN) ELISA

Catalog Number: EDN39-K01

96 Wells

For Research Use Only.

v. 2.0 (09.08.22)

Eagle Biosciences, Inc.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 866-419-2019 Fax: 617-419-1110
www.EagleBio.com



INTENDED USE

The Eagle Biosciences Eosinophil Derived Neurotoxin (EDN) ELISA Kit is intended for the quantitative determination of eosinophil derived neurotoxin (EDN) in stool, plasma, serum and urine. The EDN ELISA is for Research Use Only and is not for use in diagnostic procedures.

INTRODUCTION

After activation eosinophil granulocytes segregate 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 superfamily¹⁻³. EDN, however, has a 100-fold increased ribonuclease activity. EDN is also neurotoxic and not cytotoxic^{4,5}. The activation of eosinophil granulocytes is important during the inflammatory processes in allergic reactions. Thus EDN is a marker for eosinophil activation and degranulation.

The measurement of EDN in stool allows the detection of clinical or subclinical chronic inflammation in the gut. Some research has suggested that the measurement of EDN gives information on the activity of disease and the prediction of a relapse. The Eosinophil Derived Neurotoxin (EDN) ELISA Kit allows an easy, rapid and precise quantitative determination of the eosinophil derived neurotoxin in biological samples. The kit includes all reagents ready to use for preparation of the samples.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are strictly intended for Research Use Only.
- Do not interchange kit components from different lots.
- The stop solution (STOP) contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.
- The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill should be wiped out immediately with copious quantities of water.
- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- The reagents of the Eosinophil Derived Neurotoxin (EDN) ELISA Kit contain bactericides to protect against bacterial growth. Avoid the contact with the skin or mucous membrane.
- Reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.



MATERIALS PROVIDED

Catalog #	Component	Description	Amount
W 2000mtp	MTP	Microtiter Plate coated	12 x 8 wells
W 2000vp	SAMPLEBUF	Sample buffer conc. 10 fold	100 ml
W 2000wp	WASHBUF	ELISA wash buffer conc. 10 fold	100 ml
W 2000ap	ASSAYBUF	Assay buffer	50 ml
W 2000st	STD	Standard	7 vials
W 2000ko	CTRL-pos CTRL-neg	Control (positive and negative)	1 vial each
W 2000kg	CONJ	Conjugate, polyclonal peroxidase- labelled antibody	12 ml
W 2000su	SUB	TMB substrate (tetramethylbenzidine)	20 ml
W 2000sp	STOP	Stop solution	20 ml

MATERIALS NEEDED BUT NOT PROVIDED

- Laboratory balance
- Centrifuge, 3000xg
- Glass or plastic vials
- Various pipettes
- Foil to cover the microtiter plate
- Multichannel or multi-pipette
- ELISA reader with filter 450 nm (reference filter 620 or 690 nm)
- Microtiter plate shaker
- Vortex mixer

REAGENT PREPARATION

Microtiter Plate (MTP): Take the needed strips out of the bag and mount them on the holder. Please take care that the package has reached room temperature before opening the bag. Strips which are not needed could be stored at 2-8°C. Please dispose the holder when all strips are used.

Wash Buffer (WASHBUF): Dilute the wash buffer concentrate 1:10 with deionized or distilled water (1 part buffer + 9 parts water). The dilution is stable for 14 days at 2-8°C.
Important: When storing the wash buffer concentrate at 2-8°C crystallization could occur. Before dilution all crystals must be dissolved.



Sample Buffer (SAMPLEBUF): Dilute the sample buffer concentrate 1:10 with deionized or distilled water (1 part buffer + 9 parts water). The dilution is stable for 14 days at 2-8°C. *Important: When storing the sample buffer concentrate at 2-8°C crystallization could occur. Before dilution all crystals must be dissolved.*

It is recommended to dilute only the amount of buffer which is used to process the given samples. All other test reagents are stable at 2-8 °C, up to the date of expiry stated on the label.

SPECIMEN

Stool Samples

Eosinophil derived neurotoxin (EDN) is extracted by the sample dilution buffer out of the stool sample.

- 100 mg stool are mixed with 5 ml SAMPLEBUF on a vortex mixer until the mixture is homogenous.
- 1 ml of the mixture is transferred into an "Eppendorf" reaction vial and centrifuged for 30 min at 3000xg (5 min at 10000xg) and incubated over night at 2-8 °C.
- 100 µl of the supernatant are used in the test per well.

Serum and Plasma Samples

- Serum samples are allowed to clot for 60 min at 18-25°C and centrifuged afterwards. Plasma samples are centrifuged within 30 min. The supernatant is transferred to a new vial.
- 50 µl sample + 200 µl ASSYBUF are mixed on a vortex mixer
- 100 µl are used in the test per well

Urine Samples

We recommend using a 24h urine sample. The daily EDN excretion is mentioned in mg/day. If a 24h urine is not available, the sample can be related on the creatinine clearance and processed as "mg EDN/mmol creatinine".

- 10 µl sample + 500 µl ASSYBUF are mixed on a vortex mixer
- 100 µl are used in the test per well

PROCEDURE

Principle of The Assay

The Eosinophil Derived Neurotoxin (EDN) ELISA Kit determines human EDN according to the "sandwich" ELISA principle. EDN in sample, standard and controls binds to monoclonal 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.

SAMPLE PREPARATION

All reagents and samples should be at room temperature (18-26°C) and mixed well before use.



The position of standards, controls and samples should be noted.

1. Sample Incubation

- Pipette **100µl STD** (undiluted), **CTRL** (1:5 diluted with ASSYBUF) and **samples** in duplicate to the microtiter plate. For standard 0 use the corresponding dilution buffer (See Section 12) of the samples
- The strips are covered and incubated by shaking for **60 min** at room temperature (18-26 °C).
- The reaction in each well starts immediately. Pipetting should be performed as quickly as possible. When processing many samples at once the samples should be pipetted to a separate microtiter plate (150 µl) and transferred simultaneously using a multichannel pipette.

2. Washing Step

- Discard the contents of the microwells and wash 5x with 250 µl diluted WASHBUF per well. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

3. Incubation Conjugate

- Pipette **100 µl CONJ** in each microwell.
- The strips are covered and incubated by shaking at room temperature (18-26 °C) for **60 min**

4. Washing Step

- Discard the contents of the microwells and wash 5x with 250 µl diluted WASHBUF per well. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

5. Incubation Substrate

- Pipette **100 µl SUB** in each microwell.
- Incubate for **5-10 min** at room temperature (18-26 °C) in the dark.

6. Stopping Reaction

- Pipette **100 µl STOP** in each microwell, mix well.

7. Reading

- Read the absorbance at 450 nm. If the microtiter plate reader allows to use a reference wavelength use 620 or 690 nm as reference wavelength.
- Reading should be done within 30 min after stopping reaction.
- In case that the highest standard exceeds the range of the reader the reading should be done at 405 nm against 620 nm (690 nm).



CALCULATIONS

For calculating the results we recommend to use the 4-parameter algorithm. If this algorithm is not available, a "point to point" or a "spline" function can be used.

Stool Samples

- The obtained EDN concentration is multiplied by 50

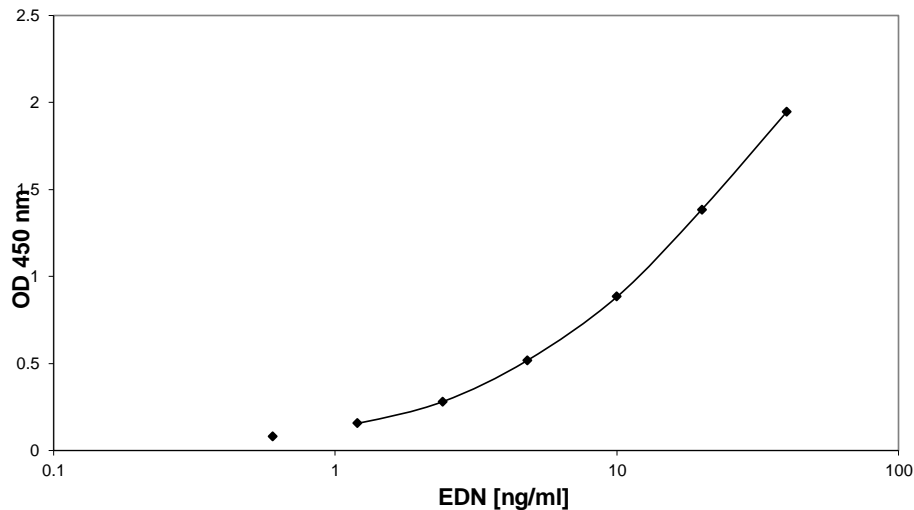
Serum/Plasma Samples

- The obtained EDN concentration is multiplied by 5

Urine Samples

- The obtained EDN concentration is multiplied by 51

Typical Standard Curve



The curve given above is only for demonstration. It must not be used for calculation of your samples

INTERNAL QUALITY CONTROL

Reference Values

- Stool (n= 87): preliminary cut off 360 ng/ml
In a study the EDN concentration was measured in 87 probands and the preliminary cut off value was found.
- Urine (n=50): 81.8 (26.7 – 164.2) µg/mmol creatinine
- Serum (n=52): 26,4 (8,3 – 66,4) ng/ml
- Plasma (n=52): 18,1 (6,2 – 49,8) ng/ml



We recommend that each laboratory develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

VALIDATION DATA

Precision and Reproducibility

Intra-Assay CV stool:	2.2 % (1966 ng/ml)	[n = 12]
Inter-Assay CV stool:	9.5 % (819 ng/ml)	[n = 6]
Intra-Assay CV serum:	3.5 % (3.5 ng/ml)	[n = 9]
Inter-Assay CV serum:	7.2 % (4.2 ng/ml)	[n = 4]

Linearity:

Stool	up to 2000 ng/ml
Serum	up to 22 ng/ml

Detection Limit:

Stool	6.0 ng/ml
Serum	0.62 ng/ml

Recovery:

Stool	102.4 %
Serum	92 %

LIMITATIONS

Serum/plasma and urine samples with EDN concentrations above the standard curve should be diluted with assay buffer (ASSYBUF) and measured again.

Stool samples with EDN concentrations above the standard curve should be diluted with sample dilution buffer (SAMPLEBUF) and measured again.

DISPOSAL

The substrate (SUB) must be disposed as non-halogenated solvent. The stop solution (STOP) could be neutralized with NaOH and if the pH value is neutral it can be disposed as salt solution. **(Important:** Reaction will produce heat, be careful)

Please refer to the appropriate national guidelines.

REFERENCES

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.