

Product information



User's Manual

EDN ELISA

For the determination of EDN (eosinophil-derived neurotoxin) in stool, urine, serum and plasma.

For research use only, not for use in diagnostic procedures.

REF

ID09031



96

Storage: 2–8°C

Manufactured for:

Immuno-Biological Laboratories, Inc. (IBL-America)

8201 Central Ave NE, Suite P, Minneapolis, MN 55432

Toll Free: (888) 523-1246 Fax: (763) 780-2988

www.ibl-america.com / info@ibl-america.com

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1. INTENDED USE

The described ELISA is intended for the determination of EDN (eosinophil-derived neurotoxin, eosinophil protein x, EPX) in serum, plasma, urine and stool. For research use only, not for use in diagnostic procedures.

2. INTRODUCTION

EDN (eosinophil-derived neurotoxin, eosinophil protein x, EPX), a cationic glycoprotein, which is released by activated eosinophils, has strong cytotoxic characteristics and plays a significant role in the prevention of virus infections. It is released by the eosinophil granules in places where eosinophils are mainly found: in the skin, lungs, urogenital and gastrointestinal tract, that is, in the organs acting as an entry point for pathogens. The accumulation of EDN in the intestine is associated with inflammation and tissue damage.

Measuring of EDN in stool can serve as an objective parameter for a current chronic inflammation located in the gastrointestinal area. In the case of Colitis ulcerosa and Crohn's disease, the EDN measurement enables the evaluation of a disease's activity and the prediction of a relapse.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6821MTP	PLATE	Holder with precoated strips	12 x 8 wells
K 6821WP	WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
K 6821EP	IDK Extract®	Extraction buffer concentrate <i>IDK Extract®</i> 2.5x	2 x 100 ml
K 6821AP	ASYBUF	Assay buffer, ready to use	50 ml
K 6821CAL	CAL	Calibrator, lyophilized	2 x 1 vial
K 6821KO1	CTRL	Control, lyophilized (see specification for range)	2 x 1 vial

Cat. No.	Label	Kit components	Quantity
K 6821KO2	CTRL	Control, lyophilized (see specification for range)	2 x 1 vial
K6821K	CONJ	Conjugate, polyclonal peroxidase-labeled antibody	200 µl
K 6821TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	15 ml
K 6821AC	STOP	ELISA stop solution, ready to use	15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* IBL-America recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted **1:10 in ultra pure water** before use (100 ml concentrate + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37 °C before dilution of the buffer solutions. The **buffer concentrate** is stable at **2–8 °C** until the expiry date stated

on the label. **Diluted buffer solution** (wash buffer) can be stored in a closed flask at **2–8 °C for one month**.

- The **extraction buffer concentrate *IDK Extract***[®] must be diluted with ultra pure water **1:2.5** before use (100 ml EXBUF + 150 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. Before dilution, the crystals must be redissolved at 37°C in a water bath. The **extraction buffer concentrate *IDK Extract***[®] is stable at **2–8 °C** until the expiry date stated on the label. Diluted **buffer solution** (extraction buffer) can be stored in a closed flask at **2–8 °C for three months**.
- The **lyophilized CAL** (calibrator) and **CTRL** (controls) are stable at **2–8°C** until the expiry date stated on the label. The CAL (calibrator) and **CTRL** (controls) must be reconstituted with **500 µl** ultra pure water. Allow the vial content to dissolve for **10 minutes** and mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted calibrators and controls can be stored at 2–8°C for four weeks**.
- The **conjugate concentrate** (CONJ) must be diluted **1:101 in wash buffer** (100 µl CONJ + 10 ml wash buffer). The undiluted conjugate is stable at **2–8 °C** until the expiry date stated on the label. **Diluted conjugate is not stable and cannot be stored**.
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

The stool sample stability is as follows:

Raw stool can be stored for 72 hours at room temperature (15-30 °C) and 4 °C or for 8 weeks at -20 °C.

Stool extracts (1:100) can be stored for 1 day at room temperature (15–30 °C), for 5 days at 2–8°C or for seven days at -20 °C. Avoid more than two freeze-thaw cycles.

Extraction of the stool samples

Diluted extraction buffer *IDK Extract*[®] is used as a sample extraction buffer. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 1.5 ml extraction buffer:

Applied amount of stool: 15 mg

Buffer Volume: 1.5 ml

Dilution Factor: 1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

- a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenization using an applicator, inoculation loop or similar device.
- b) Fill the **empty sample tube** with **1.5 ml** of ready-to-use *IDK Extract®* extraction buffer before using it with the sample. Important: Allow the extraction buffer to reach room temperature.
- c) Unscrew the tube (orange part of cap) to open. Insert the orange dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.
- d) Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for ~ 10 minutes improves the result.
- e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
- f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:100

Dilution of samples

Stool samples

The supernatant of the extraction (dilution I) is diluted **1:4** with **wash buffer**. For example:

100 µl dilution I + **300 µl** wash buffer = **dilution II**

(1:4) **Final dilution: 1:400***

* A dilution of 1:1000 is recommended for sample collectives with expected elevated values.

For analysis, pipet **100 µl of dilution II** per well.

Urine samples

We recommend to analyze urine collected within **24 hours**, whereby the **EDN concentration** is expressed as mg/day. If a 24 h urine sample is not available, urine from a single time point can be analyzed. In this case, the urinary creatinine should also be determined, and the EDN results are presented as µg/mmol creatinine.

Within 30 min of urine collection, the urine is separated by centrifugation, twice for 10 min at 1350 g and 4 °C. The supernatant is then transferred to a new plastic tube.

Prior to analysis, the urine samples should be diluted **1:400** with ASYBUF (assay buffer).

For example:

10 µl sample + **190 µl** ASYBUF = **dilution I (1:20)**

15 µl dilution I + **285 µl** ASYBUF = **dilution II (1:20)**

Final dilution: 1:400

For analysis, pipet **100 µl of dilution II** per well.

Serum/plasma samples

Fresh collected serum/plasma should be centrifuged within one hour. Store samples at -20 °C if not assayed on the same day. Lipemic or hemolytic samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicate analyses for each sample.

The serum/plasma samples should be diluted 1:40 with ASYBUF (assay buffer), prior to analysis.

10 µl sample + **390 µl** ASYBUF

Final dilution: 1:40

For analysis, pipet **100 µl of the dilution** per well.

7. ASSAY PROCEDURE

Principle of the test

The assay utilizes the two-site sandwich ELISA technique with two selected antibodies (monoclonal and polyclonal) that bind to human EDN.

Calibrator, controls and diluted samples which are assayed for EDN are added to wells of microplate coated with a high affine monoclonal anti-human EDN antibody. During the first incubation step, EDN in the samples is bound by the immobilized antibody. Then a peroxidase-labeled conjugate is added to each well and the following complex is formed: capture antibody - human EDN - peroxidase conjugate. Tetramethylbenzidine is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the EDN concentration of the sample. Samples are determined by referring their optical density to a lot-dependent master calibration curve and the use of the calibrator that is run with each test.

Test procedure

Prior to use in the assay, allow all reagents and samples to come to room temperature (15–30 °C) and mix well.

Mark the positions of BLANK/CAL/SAMPLE/CTRL (blank/calibrator/sample/controls) on a protocol sheet.

Take the microtiter strips out of the kit. Store unused strips covered at 2–8 °C. Strips are stable until the expiry date stated on the label.

We recommend to carry out the tests in duplicate.

1.	Wash each well 5 times by dispensing 250 µl of diluted WASHBUF (wash buffer) into each well. After the final washing step, remove residual buffer by tapping the plate on absorbent paper.
2.	Add 100 µl of BLANK/CAL/SAMPLE/CTRL (blank/calibrator/sample/controls) into respective well.
3.	Cover the plate tightly and incubate for 1 hour at room temperature (15–30 °C) on a horizontal mixer.
4.	Discard the contents of each well. Wash 5 times by dispensing 250 µl of diluted WASHBUF (wash buffer) into each well. After the final washing step, remove residual buffer by tapping the plate on absorbent paper.
5.	Add 100 µl conjugate into each well.

6.	Cover the plate tightly and incubate for 1 hour at room temperature (15–30 °C) on a horizontal mixer.
7.	Discard the contents of each well. Wash 5 times by dispensing 250 µl of diluted WASHBUF (wash buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
8.	Add 100 µl of SUB (substrate) into each well.
9.	Incubate for 10–20 minutes at room temperature (15–30 °C) in the dark*.
10.	Add 100 µl of STOP (stop solution) into each well, mix thoroughly.
11.	Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

* The intensity of the color change is temperature sensitive. We recommend observing the color change and stopping the reaction upon good differentiation.

8. RESULTS

For result evaluation, please use a four parametric logit-log model based on the standard curve of the respective kit lot and the calibrator value (CAL). All essential information on the standard curve is provided on the QC data sheet of the respective product lot.

The calibration curve can be expressed either by the concentration of each standard with its corresponding optical density or by the four parameters A,B,C and D. In both cases the optical density of the calibrator (CAL) is essential. Depending on your evaluation software program, either the one or the other kind of data described above should be entered.

Caution: Please make sure that all parameters and values are transferred accurately into your software as minor deviations can cause severe errors during evaluation.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

Stool and urine samples

For the calculation of the EDN concentration in stool and urine samples, the

result must be multiplied by the dilution factor 400 or by 1000 when a dilution of 1:1000 has been used.

Serum/plasma samples

For the calculation of the EDN concentration in plasma/serum the result must be multiplied by the dilution factor 40.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range must be further diluted and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly determined.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

10. QUALITY CONTROL

IBL-America recommends the use of external controls for internal quality control, if possible.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference ranges

1 g stool is equivalent to 1 ml.

Stool (n = 53): 357.6 ng/ml

Based on studies of evidently healthy persons (n = 53). a mean value of 357.64 ng/ml stool was estimated. For the present. the value of 1700 ng/ml stool should be considered as the upper limit of the test.

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 each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Two samples measured using the assay. **Intra-**

Assay (n = 23)

Sample	EDN [ng/ml]	CV [%]
1	303.6	7.0
2	760.5	5.7

Inter-Assay (n = 14)

Sample	EDN [ng/ml]	CV [%]
1	378.6	9.5
2	722.9	6.2

Analytical Sensitivity

The Zero-standard was measured 21 times. The detection limit was set as $B_0 + 2$ SD and estimated to be 0,164 ng/ml.

Spiking Recovery

Two samples were spiked with 4 different EDN standards and measured using this assay (n = 2).

Sample	Unspiked sample [ng/ml]	Spike [ng/ml]	EDN expected [ng/ml]	EDN measured [ng/ml]
A	0.672	1.50	2.172	2.181
	0.672	2.00	2.672	2.546
	0.672	2.50	3.172	2.962
	0.672	4.00	4.672	4.551

Sample	Unspiked sample [ng/ml]	Spike [ng/ml]	EDN expected [ng/ml]	EDN measured [ng/ml]
B	1.294	0.50	1.794	1.994
	1.294	1.50	2.794	3.156
	1.294	2.00	3.294	3.674
	1.294	3.50	4.794	5.284

Dilution recovery

Two samples were diluted and analyzed. The results are shown below (n = 2):

Sample	Dilution	EDN expected [ng/ml]	EDN measured [ng/ml]
A	1:200	798.10	798.10
	1:400	399.05	451.30
	1:800	199.53	231.10
	1:1600	99.76	109.40
B	1:200	281.20	281.20
	1:400	140.60	175.40
	1:800	70.30	85.10
	1:1600	35.15	32.30

12. PRECAUTIONS

- All reagents in the kit package are for research use only, not for use in diagnostic procedures.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any

spill should be wiped up immediately with copious quantities of water. Do not breath vapor and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. IBL-America can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to IBL-America along with a written complaint.

15. REFERENCES

1. Konikoff, M. R. et al. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. *Clin. Gastroenterol. Hepatol.* **4**, 1328–36 (2006).
2. Lotfi, R. & Lotze, M. T. Eosinophils induce DC maturation, regulating immunity. *J. Leukoc. Biol.* **83**, 456–60 (2008).
3. Bentz, S. et al. Clinical relevance of IgG antibodies against food antigens in Crohn's disease: a double-blind cross-over diet intervention study. *Digestion* **81**, 252–64 (2010).
4. Kalach, N. et al. Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clin. Chem. Lab. Med.* **51**, 351–61 (2013).

Used symbols:



Temperature limitation



Catalogue Number



In Vitro Diagnostic Medical Device



Contains sufficient for <n> tests



Manufacturer



Use by



Lot number