




Instructions for Use

EDN (EPX) ELISA

IVD



REF EIA-6111

 96



DRG 

DRG Instruments GmbH, Germany
Frauenbergstraße. 18, 35039 Marburg
Phone: +49 (0)6421-1700 0, Fax: +49 (0)6421-1700 50
Website: www.drg-diagnostics.de
E-mail: drg@drg-diagnostics.de

Distributed by:

DRG 

DRG International, Inc., USA
841 Mountain Ave., Springfield, NJ 07081
Phone: (973) 564-7555, Fax: (973) 564-7556
Website: www.drg-international.com
E-mail: corp@drg-international.com

***Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.***

Table of Contents

1	INTENDED USE.....	2
2	INTRODUCTION.....	2
3	MATERIAL SUPPLIED.....	2
4	MATERIAL REQUIRED BUT NOT SUPPLIED.....	2
5	STORAGE AND PREPARATION OF REAGENTS.....	3
6	STORAGE AND PREPARATION OF SAMPLES.....	4
7	ASSAY PROCEDURE.....	6
8	RESULTS.....	7
9	LIMITATIONS.....	7
10	QUALITY CONTROL.....	7
11	PERFORMANCE CHARACTERISTICS.....	8
12	PRECAUTIONS.....	9
13	TECHNICAL HINTS.....	9
14	GENERAL NOTES ON THE TEST AND TEST PROCEDURE.....	9
15	REFERENCES / LITERATURE.....	9
	SYMBOLS USED.....	10

1 INTENDED USE

The described ELISA is intended for the quantitative determination of EDN (eosinophil-derived neurotoxin, also known as RNASE2 or eosinophil protein x [EPX]) in serum, plasma, urine and stool.

It is for *in vitro* diagnostic use only.

2 INTRODUCTION

EDN (eosinophil-derived neurotoxin, eosinophil protein x, EPX), a cationic glycoprotein, which is released by activated eosinophiles, has strong cytotoxic characteristics and plays a significant role in the prevention of virus infections. It is released by the eosinophile granules in places where eosinophiles 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 clinical or sub-clinical 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.

Indications

- Morbus Crohn
- Proof of a food allergy and incompatibility
- Assessment of an elimination diet
- Proof of damaged integrity of the intestinal mucous membrane (e. g. chronic inflammatory bowel disease, colon cancer)
- Proof of intestinal parasites / parasitoses

3 MATERIAL SUPPLIED

Label	Kit components	Quantity
PLATE	Holder with precoated strips	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 mL
IDK Extract[®]	Extraction buffer concentrate <i>IDK Extract[®] 2.5x</i>	2 x 100 mL
ASYBUF	Assay buffer, ready to use	50 mL
STD	Standard, lyophilized	2 x 5 vials
CTRL 1	Control, lyophilized (see specification for range)	2 x 1 vial
CTRL 2	Control, lyophilized (see specification for range)	2 x 1 vial
CONJ	Conjugate, polyclonal peroxidase-labeled antibody	200 µL
SUB	TMB substrate (Tetramethylbenzidine), ready to use	15 mL
STOP	ELISA stop solution, ready to use	15 mL

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Laboratory balance
- Calibrated precision pipettors and 10 µL - 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)

* DRG recommends the use of Ultrapure 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.
- **Preparation of the wash buffer:**
The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 mL WASHBUF + 900 mL ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2 °C - 8 °C** until the expiry date stated on the label.
Wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at **2 °C - 8 °C for 1 month**.
- **Preparation of the extraction buffer:**
The **extraction buffer concentrate *IDK Extract*[®]** must be diluted with ultrapure water **1:2.5** before use (100 mL *IDK Extract*[®] + 150 mL ultrapure 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 ***IDK Extract*[®]** is stable at **2 °C - 8 °C** until the expiry date stated on the label.
Extraction buffer (1:2.5 diluted *IDK Extract*[®]) can be stored in a closed flask at **2 °C - 8 °C for three months**.
- The **lyophilized standards (STD)** and **controls (CTRL)** are stable at **2 °C - 8 °C** until the expiry date stated on the label. Before use, the **STD** (standards) and **CTRL** (controls) must be reconstituted with **500 µL of ultrapure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to ensure complete reconstitution. **Standards and controls** (reconstituted STDs and CTRLs) **can be stored at 2 °C - 8 °C for 4 weeks**.
- **Preparation of the conjugate:**
The **conjugate concentrate (CONJ)** must be diluted **1:101** in wash buffer (100 µL CONJ + 10 mL wash buffer). The CONJ is stable at **2 °C - 8 °C** until expiry date stated on the label.
Conjugate (1:101 diluted CONJ) **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 °C - 8 °C**.

6 STORAGE AND PREPARATION OF SAMPLES

6.1 Stool samples

6.1.1 Sample storage

Raw stool can be stored for 72 hours at room temperature (15 °C - 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 °C - 30 °C), for 5 days at 2 °C - 8 °C or for seven days at -20 °C. Avoid more than two freeze-thaw cycles.

6.1.2 Extraction of the stool samples

Extraction buffer (1:2.5 diluted *IDK Extract*[®]) is used as a sample extraction buffer. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: EIA-5674)

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:

- The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.
- Fill the **empty sample tube** with **1.5 mL extraction buffer** (1:2.5 diluted *IDK Extract*[®]) before using it with the sample. Important: Allow the extraction buffer to reach room temperature.
- 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.
- 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.
- Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
- 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

6.1.3 Dilution of 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.

6.2 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 quantified, and the EDN results are presented as $\mu\text{g}/\text{mmol}$ 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.

6.3 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

7.1 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.

Assay standards, controls and prediluted patient samples containing human EDN are added to wells of microplate that was coated with a high affine monoclonal anti-human EDN antibody. After the first incubation period, antibody immobilized on the wall of microtiter wells captures human EDN in the sample. Then a peroxidase-conjugated rabbit polyclonal anti-human EDN antibody is added to each microtiter well and a sandwich of capture antibody – human EDN – Peroxidase-conjugate is formed. 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 concentration of EDN. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. EDN present in the patient samples is determined directly from this curve.

7.2 Test procedure

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

Mark the positions of standards/samples/controls on a protocol sheet.

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

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or DRG.

We recommend to carry out the tests in duplicate.

1. Wash the pre-coated microtiter plate **5 x with 250 µL wash buffer before use**.
After the final washing step, the inverted microtiter plate should be tapped on absorbent paper.
2. Add each **100 µL standards/samples/controls** into the respective wells.
3. Cover the plate tightly and incubate for **1 hour** at room temperature (15 °C - 30 °C) **on a horizontal mixer**.
4. Discard the contents of each well and wash **5 times** with **250 µL wash buffer**.
After the final washing step, the inverted microtiter plate should be firmly tapped 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 °C - 30 °C) **on a horizontal mixer**.
7. Discard the contents of each well and wash **5 times** with **250 µL wash buffer**.
After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8. Add **100 µL substrate** (SUB) in each well.
9. Incubate for **10 - 20 min** at room temperature (15 °C - 30 °C) **in the dark***.
10. Add **100 µL stop solution** (STOP) 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

The following algorithms can be used alternatively to calculate the results. We recommend using the “4 parameter algorithm”.

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the paired 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 (see definition below) must be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

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

$$\text{highest concentration of the standard curve} \times \text{sample dilution factor to be used}$$

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

$$\text{LoB} \times \text{sample dilution factor to be used}$$

10 QUALITY CONTROL

DRG recommends the use of external controls for internal quality control, if possible.

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

10.1 Reference ranges

1 g stool is equivalent to 1 mL.

Stool (n = 53): 357.6 ng/mL (mean value)

Based on DRG studies of evidently healthy persons (n = 53), a mean value of 357.64 ng/mL stool (standard deviation: 500.1 ng/mL) was estimated. The mean value + 2 SD (= 1357.8 ng/mL) should be considered as the preliminary 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

11.1 Precision and reproducibility

Two patient samples were measured using the EDN (EPX) ELISA.

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

11.2 Analytical Sensitivity

The Zero-standard was measured 21 times. The detection limit was set as B0 + 2 SD and estimated to be 0.164 ng/mL.

11.3 Specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to EDN. The specificity is calculated in percent, based on the cross-reactivity of these compounds with the anti-EDN antibody compared to the EDN antigen:

Lactoferrin	0 %
PMN Elastase	0 %
sIgA	0 %
Albumin	0 %

11.4 Spiking Recovery

Two samples were spiked with 4 different EDN standards and measured using the EDN (EPX) ELISA (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
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

11.5 Dilution recovery

Two patient 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	451.30	399.05
	1:800	231.10	199.53
	1:1600	109.40	99.76
B	1:200	281.20	281.20
	1:400	175.40	140.60
	1:800	85.10	70.30
	1:1600	32.30	35.15

12 PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- 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 vapour 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 colourless 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. DRG can therefore not be held responsible for any damage resulting from incorrect use.

15 REFERENCES / LITERATURE

1. Konikoff, M.R. et al., 2006. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*, **4**(11), pp.1328–36.
2. Lotfi, R. & Lotze, M.T., 2008. Eosinophils induce DC maturation, regulating immunity. *Journal of leukocyte biology*, **83**(3), pp.456–60.
3. Bentz, S. et al., 2010. Clinical relevance of IgG antibodies against food antigens in Crohn's disease: a double-blind cross-over diet intervention study. *Digestion*, **81**(4), pp.252–64.
4. Kalach, N. et al., 2013. Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clinical chemistry and laboratory medicine : CCLM / FESCC*, **51**(2), pp.351–61.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
	<i>In vitro</i> diagnostic medical device *	<i>In-vitro</i> -Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
	Catalogue number *	Artikelnummer *	Numero di Catalogo	Número de catálogo	Référence de catalogue
	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
<i>Distributed by</i>	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
<i>Content</i>	Content	Inhalt	Contenuto	Contenido	Contenu
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité