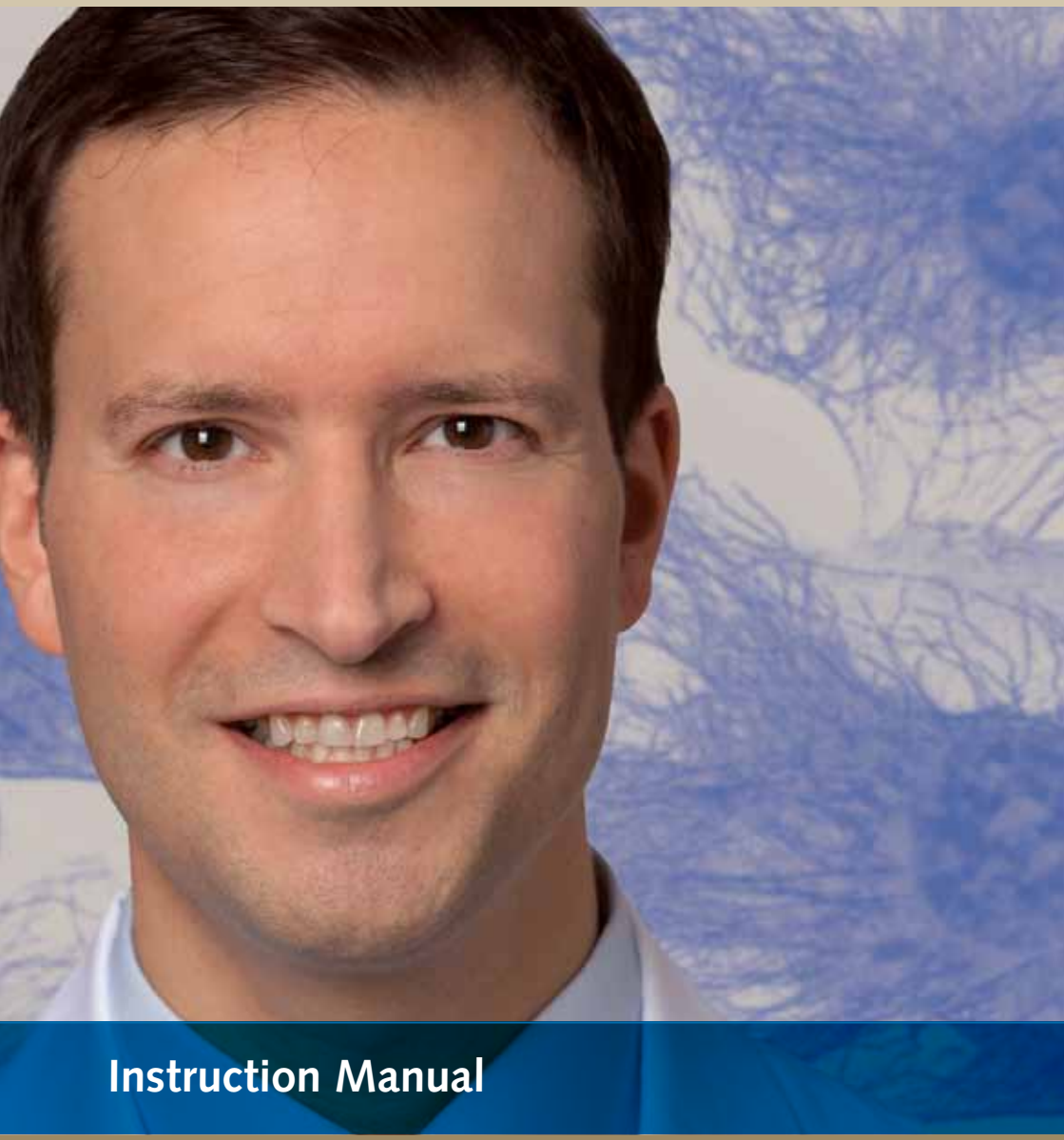
Human EDN (EPX) ELISA Kit



Instruction Manual

PromoKine

Contents

Intended Use

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the quantitative determination of EDN (eosinophil-derived neurotoxin, eosinophil protein x, EPX) in serum, plasma, urine and stool. **It is for research use only.**

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 caused by an invasive disease (e.g. CED, *CC* etc.)
- Proof of intestinal parasites
- Parasitoses

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
EXBUF	Extraction buffer concentrate 2.5x	2 x 100 ml
ASYBUF	Assay buffer, ready-to-use	50 ml
STD	Standard, lyophilized (see specification for range)	2 x 5 vials
CTRL1	Control 1, lyophilized (see specification for range)	2 x 1 vial
CTRL2	Control 2, lyophilized (see specification for range)	2 x 1 vial
CONJ	Conjugate, ploYclonal peroxidase-labeled antibody	0.2 ml
SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	15 ml
STOP	ELISA stop solution, ready-to-use	15 ml

Material Required But Not Supplied

- Ultra-pure water*
- Laboratory balance
- Calibrated precision pipettors and disposable tips for 5-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeater pipets
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.

- Microtiter plate reader at 450 nm (reference wave length 620 or 690 nm)

* Ultra-pure water (Water Type I; ISO3696) which is free of undissolved and colloidal ions and organic molecules (free of particles >0.2 µm) with an electric conductivity <0.055 µS/cm at 25°C (≥18.2 MΩ cm) is recommended.

Preparation and Storage 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** (EXBUF) must be diluted with ultra-pure water **1:2.5** before use (100 ml concentrate + 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** 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 four months**.
- The **lyophilized standards** (STD) and **controls** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. Before use, the **STD** (standards) and **CTRL** (controls) must be reconstituted with **500 µl of ultra pure water**. Allow the vial content to dissolve for 10minutes and mix thoroughly by gentle inversion to ensure

complete reconstitution. **Reconstituted standards and controls can be stored at 2–8 °C for four weeks.**

- The **conjugate** (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**.

Sample Preparation

Sample storage

The stool sample stability is as follows:

Crude stool: 72 hours at room temperature (18–26 °C) and 4 °C; for 8 weeks at -20 °C.

Stool extracts (1:50): 48 hours at 4°C and 72 hours at -20 °C.

Extraction of the stool samples

Diluted extraction buffer is used as a sample extraction buffer. We recommend the following sample preparation:

Stool Sample Application System (SAS) (available at Immundiagnostik, Bensheim Cat. No.: K6998SAS)

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: 0.75 ml

Dilution Factor: 1:50

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 homogenisation using an applicator, inoculation loop or similar device.

b) Fill the **empty sample tube** with **0.75 ml** of ready-to-use **extraction buffer** before using it with the sample. Important: Allow the extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow 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: 1:50

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: 1:200*

* 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 hour 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 µg/mmol creatinine.

Within 30 minutes of urine collection, the urine is separated by centrifugation, twice for 10 minutes at 1,350 g and 4 °C. The supernatant is then transferred to a new plastic tube.

Prior to analysis, the urine samples should be diluted **1:200** with ASYBUF (assay buffer).

For example:

10 µl sample + **90 µl** ASYBUF = **dilution I** (1:10)
15 µl dilution I + **285 µl** ASYBUF = **dilution II** (1:20)
Final dilution: 1:200

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 1:20 with ASYBUF (assay buffer), prior to analysis.

20 µl sample + **380 µl** ASYBUF
Final dilution: 1:20

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

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.

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.

Test procedure

We recommend to carry out the tests in duplicate.

1.	Prior to use in the assay, allow all reagents and samples to come to room temperature (15–30 °C) and mix well.
2.	Mark the positions of STD /SAMPLE/CTRL (standards/sample/controls) on a protocol sheet.
3.	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.
4.	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.
5.	Add **100 µl of STD/SAMPLE/CTRL** (standard/sample/controls) into respective 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 conjugate** into each well.
9.	Cover the plate tightly and incubate for **1 hour** at room temperature (15–30 °C) on a horizontal mixer.
10.	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.
11.	Add **100 µl of SUB** (substrate) into each well.
12.	Incubate for **10–20minutes** at room temperature (15–30 °C) in the dark*.
13.	Add **100 µl of STOP** (stop solution) into each well, mix thoroughly.
14.	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 to observe the color change and to stop the reaction upon good differentiation.

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 has to be specified with a value smaller 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 program 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 200 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 20.

Limitations

Stool samples with an OD higher than the OD of the highest standard, should be diluted with sample dilution buffer and re-assayed.

Serum/plasma and urine samples with an OD higher than the OD of the highest standard, should be diluted with sample dilution buffer and re-assayed.

Quality Control

PromoCell 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 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.

Reference ranges

Normal 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 norm concentration range.

Performance Characteristics

Precision and reproducibility

Two patient samples were 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

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
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 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

Precautions

- For research use only.
- The quality control guidelines should be followed.
- Human material used in the kit components was 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.
- Reagents of the kit package contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

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.
- Reagents should not be used beyond the expiration date shown on the 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.
- The assay should always be performed according the enclosed manual.

General Notes on the Test and Test Procedure

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for research use only.
- 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. PromoCell can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product should be send to PromoCell together with a written complaint.

Ordering Information

Product Name	Product Description	Size	Catalog Number
EDN (EPX) ELISA Kit, human	Human Eosinophil Derived Neurotoxin (EPX) ELISA Kit	96 Tests	PK-EL-K6810

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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