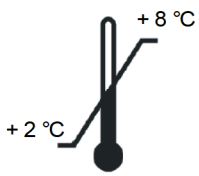
EDN ELISA Kit

For the in vitro Determination of EDN (Eosinophil-derived neurotoxin) in stool, urine, serum and plasma

Valid from 23.03.2011

K 6811



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

3 MATERIAL SUPPLIED

Catalogue No	Content	Kit Components	Quantity
K 6811MTP	PLATE	One holder with precoated strips	12 x 8 wells
K 6811WB	WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
K 6811EP	EXBUF	Extraction buffer concentrate 2.5x	2 x 100 ml
K 6811AP	ASYBUF	Assay buffer, ready-to-use	50 ml
K 6811ST	STD	Standard, lyophilized	2 x 5 vials
K 6811KO1	CTRL	Control, lyophilized (see specification for range)	2 x 1 vial
K 6811KO2	CTRL	Control, lyophilized (see specification for range)	2 x 1 vial
K6811K	CONJ	Conjugate, polyclonal peroxidase-labeled antibody	200 µl
K 6811TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	15 ml
K 6811AC	STOP	ELISA stop solution, ready to use	15 ml

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm

*Immundiagnostik AG 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 0,055µS/cm at 25°C (≤18,2MΩ cm).

5. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than one time, make sure that the reagents are stored at the conditions stated on the label. **Prepare just the appropriate amount necessary for the assay**. 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 with ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be dissolved at 37°C using a water bath 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** could be stored in a closed flask at **2-8°C for one month**.

- The **EXBUF** (extraction buffer) should 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. The crystals must be redissolved at 37°C in a water bath before dilution. The **buffer concentrate is stable at 2-8°C** until the expiry date stated on the label. **Diluted buffer solution** can be stored in a closed flask at **2-8°C for four months**.

- The **lyophilized STD** (standards) and **CTRL** (controls) are stable **at 2-8°C** until the expiry date stated on the label. The **STD** (standards) 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 standards and controls can be stored at 2-8°C for four weeks.**

- The **CONJ** (conjugate) must be diluted **1:100** in wash buffer (100 µl CONJ + 9900 µl 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 can not be stored.**

- All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

6. SAMPLE PREPARATION

Extraction of the stool sample

1a. Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instruction for use

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

SAS with 0.75 ml 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 remarkably inhomogeneous 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 yellow dipstick into sample. The lower part of the dipstick exhibits 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 and leave 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 app. 10 minutes improves the result.

e) Allow sample to stand for app. 10 minutes until sediment has settled down. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the turquoise ring plus the dipstick. Discard cap and dipstick. Make sure, the sediment will not be dispersed again.

1b. Sample preparation kit from Roche Diagnostics, Mannheim, Germany (Cat. No. 10 745 804 322)

Alternatively, other stool sample preparation kits (e.g. Sample preparation kit from Roche Diagnostics, Mannheim, Germany) can be used. In the Roche sample preparation kit, 100 mg of stool sample are suspended in 5 ml of extraction buffer using a vibrator mixer (e.g. Vortex mixer). Centrifugation of the suspension is recommended.

The sample suspension (1a. or 1b.) stable for 3 days at 2-8°C.

Dilution I (1a. or 1b.) **1:50**

Dilution of samples

Stool samples

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

100 µl supernatant (dilution I) + 300 µl wash buffer **(dilution II)**

Final dilution: 1 : 200*

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

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

Urine samples

We recommend to analyze urine collected within 24 hours, whereby the EDN concentration is expressed as mg/day. If 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 µg/mmol creatinine.

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

Prior to analyses, the urine samples should be diluted **1:200** with the **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, **100 µl of dilution II** is pipetted 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 analyses.

20 µl sample + **380 µl** ASYBUF.

Final dilution: 1 : 20

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

7. ASSAY PROCEDURE

Principle of the test

The assay utilizes the two-site "sandwich" 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. Tetramethyl-benzidine (TMB) 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

Prior to use in the assay allow all reagents and samples to come to room temperature (18-26 °C) and mix well
Mark the positions of STD /SAMPLE/CTRL (Standards/Sample/Controls) on a protocol sheet
Take microtiter strips out of the kit. Store unused strips covered at 2-8° C. Strips are stable until expiry date stated on the label
Wash each well 5 times by dispending 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
Add 100 µl of STD/SAMPLE/CTRL (Standard/Sample/Controls) in duplicate into respective well.
Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) on a horizontal mixer
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
Add 100 µl CONJ (conjugate) into each well

Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) on a horizontal mixer
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
Add 100 µl of SUB (substrate) into each well
Incubate for 10 - 20 minutes at room temperature (18-26°C) in the dark*
Add 50 µl of STOP (stop solution) into each well, mix thoroughly
Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used

*The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use 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. 0.01).

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 for the optical density a linear ordinate and for the concentration a logarithmic abscissa. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e. g. 0.01).

   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 used program, a control of the paired values should be done manually.

Stool and urine samples

For the calculation of the EDN concentration in **stool and urine samples**, the result must be multiplied by **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 **20**.

9. LIMITATIONS

Stool samples with EDN levels greater than the highest standard value, should be diluted with sample dilution buffer, and be re-assayed.

Serum/plasma and **urine** samples with EDN levels greater than the highest standard value, should be diluted with assay buffer and re-assayed.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of commercial control samples for internal quality control if available.

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.

Expected values

Normal ranges

(1 g stool is equivalent to 1ml)

Stool (n= 53): 357,6 ng/ml

Based on Immundiagnostik 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.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Two patient samples measured using the assay.

Intra-Assay (n=23)		
Sample	EDN [ng/ml]	VK [%]
1	303,58	6,99
2	760,50	5,71

Inter-Assay (n=14)		
Sample	EDN [ng/ml]	VK [%]
1	378,60	9,45
2	722,94	6,19

Recovery

Two samples were spiked with 4 different EDN standards and measured using this assay.

Recovery n=2

Sample [ng/ml]	Spike [ng/ml]	EDN expected [ng/ml]	EDN measured [ng/ml]
0,672	1,50	2,172	2,181
0,672	2,50	3,172	2,962
0,672	4,00	4,672	4,551
0,672	2,00	2,672	2,546
1,294	2,00	3,294	3,674
1,294	0,50	1,794	1,994
1,294	3,50	4,794	5,284
1,294	1,50	2,794	3,156

Sensitivity

The sensitivity was set as B_0 + 3 SD. The zero-standard was measured 21 times.

Sample	EDN mean value [OD]	Standard variation (SD)	Detection limit [ng/ml]
1	0,103	0,010	0,164

Linearity

Two patient samples were diluted and analyzed. The results are shown below:

n= 2

Sample	Dilution	Expected [ng/ml]	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

- For *in vitro* diagnostic 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.

13. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- 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.

14. 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 *in vitro* diagnostic use only.
- The guidelines for medical laboratories should be observed.
- 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. Immundiagnostik AG 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 shall be send to Immundiagnostik AG together with a written complaint.

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

Contains sufficient for <n> tests


Manufacturer


Use by

Lot number