



Distribuito in ITALIA da  
**Li StarFish S.r.l.**  
Via Cavour, 35  
20063 Cernusco S/N (MI)  
telefono 02-92150794  
info@listarfish.it  
www.listarfish.it

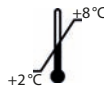
**Manual**

## **IDK<sup>®</sup> EDN ELISA**

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

Valid from 2020-01-29

**REF** **K 6821**



**IVD** **CE**



**Immundiagnostik AG**, Stubenwald-Allee 8a, 64625 Bensheim, Germany

Tel.: +49 6251 70190-0

Fax: + 49 6251 70190-363

e.mail: info@immundiagnostik.com

www.immundiagnostik.com

# Table of Contents

<b>1. INTENDED USE</b>	<b>19</b>
<b>2. INTRODUCTION</b>	<b>19</b>
<b>3. MATERIAL SUPPLIED</b>	<b>19</b>
<b>4. MATERIAL REQUIRED BUT NOT SUPPLIED</b>	<b>20</b>
<b>5. STORAGE AND PREPARATION OF REAGENTS</b>	<b>20</b>
<b>6. STORAGE AND PREPARATION OF SAMPLES</b>	<b>21</b>
<i>Sample storage</i>	21
<i>Extraction of the stool samples</i>	22
<i>Dilution of samples</i>	23
<b>7. ASSAY PROCEDURE</b>	<b>24</b>
<i>Principle of the test</i>	24
<i>Test procedure</i>	24
<b>8. RESULTS</b>	<b>25</b>
<b>9. LIMITATIONS</b>	<b>26</b>
<b>10. QUALITY CONTROL</b>	<b>26</b>
<i>Reference ranges</i>	27
<b>11. PERFORMANCE CHARACTERISTICS</b>	<b>27</b>
<i>Accuracy – Precision</i>	27
<i>Analytical sensitivity</i>	28
<i>Accuracy – Trueness</i>	28
<i>Linearity</i>	28
<i>Analytical specificity</i>	29
<b>12. PRECAUTIONS</b>	<b>29</b>
<b>13. TECHNICAL HINTS</b>	<b>30</b>
<b>14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE</b>	<b>30</b>
<b>15. REFERENCES</b>	<b>31</b>

## 1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay 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. 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

Cat. No.	Label	Kit components	Quantity
K 6821	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
K 6999.C.100	IDK Extract®	Extraction buffer concentrate IDK Extract® 2.5x	2 x 100 ml
K 6821	ASYBUF	Assay buffer, ready-to-use	1 x 50 ml
K 6821	CAL	Calibrator, lyophilised	2 x 1 vial

Cat. No.	Label	Kit components	Quantity
K 6821	CTRL 1	Control, lyophilised (see specification for range)	2 x 1 vial
K 6821	CTRL 2	Control, lyophilised (see specification for range)	2 x 1 vial
K 6821	CONJ	Conjugate concentrate, polyclonal peroxidase-labelled antibody	1 x 200 µl
K 0002.15	SUB	Substrate (Tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

#### 4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water\*
- Stool sample application system such as cat. no.: K 6998SAS
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

\* Immundiagnostik AG 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 **2–8°C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8°C for 1 month**.
- **Preparation of the extraction buffer:** The **extraction buffer concentrate IDK Extract®** has to 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 concentrate. Before dilution, the crystals have to be redissolved at 37°C in a water bath. The *IDK Extract®* is stable at **2–8°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 **2–8°C for 4 months**.
- The **lyophilised calibrator (CAL)** and **controls (CTRL)** are stable at **2–8°C** until the expiry date stated on the label. Before use, the CAL and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Calibrator and controls** (reconstituted CAL and CTRL) **can be stored at 2–8°C for 4 weeks**.
- Use **100 µl** of **wash buffer** (1:10 diluted WASHBUF) as **blank**.
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The **CONJ** is stable at **2–8°C** until the 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) 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

**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.: 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 sample 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 homogenisation using an applicator, inoculation loop or similar device.
- b) Fill the **empty stool sample tube** with **1.5 ml sample extraction buffer** (1:2.5 diluted *IDK Extract*®) before using it with the sample. **Important:** Allow the sample 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) Vortex 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 sample extraction 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)

This results in a **final dilution** of **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 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 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 assay buffer (ASY-BUF).

For example:

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

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

This results in a **final dilution** of **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 assay buffer (ASYBUF), prior to analysis.

**10 µl** sample + **390 µl** ASYBUF

This results in a **final dilution** of **1:40**.

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

## 7. ASSAY PROCEDURE

### *Principle of the test*

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

Calibrator, controls and diluted patient 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 immobilised antibody. Then a peroxidase-labelled 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 colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the EDN concentration of the sample. Samples are quantified 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 controls/calibrator/blank/samples on a protocol sheet.

Take the microtiter strips out of the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8°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 Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	<b>Before use</b> , wash the wells <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each <b>100 µl calibrator/controls/blank/prepared samples</b> into the respective wells.
3.	Cover the plate tightly and incubate for <b>1 hour</b> at room temperature (15–30°C) on a <b>horizontal shaker*</b> .



4.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add <b>100 µl conjugate</b> (diluted CONJ) into each well.
6.	Cover the plate tightly and incubate for <b>1 hour</b> at room temperature (15–30 °C) on a <b>horizontal shaker*</b> .
7.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add <b>100 µl substrate</b> (SUB) into each well.
9.	Incubate for <b>10–20 min**</b> at room temperature (15–30 °C) in the <b>dark</b> .
10.	Add <b>100 µl stop solution</b> (STOP) into each well and mix well.
11.	Determine <b>absorption immediately</b> with an ELISA reader at <b>450 nm</b> 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 <b>405 nm</b> against 620 nm as a reference.

\* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

\*\* The intensity of the colour change is temperature sensitive. We recommend observing the colour 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 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 programme used, the duplicate values should be evaluated manually.

### Stool and urine samples

The obtained results have to be multiplied by the **dilution factor 400** to get the actual concentrations, or by the **dilution factor 1000** when a dilution of 1:1000 has been used.

### Serum/plasma samples

The obtained results have to be multiplied by the **dilution factor 40** to get the actual concentrations.

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) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

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

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*

LoB see chapter "Performance characteristics".

## 10. QUALITY CONTROL

Immundiagnostik AG 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.

### Reference ranges

1 g stool is equivalent to 1 ml.

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

Based on Immundiagnostik AG 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

### Accuracy – Precision

#### Repeatability (Intra-Assay); n = 28

The repeatability was assessed with 3 stool samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [ng/ml]	CV [%]
1	1.53	7.2
2	3.73	8.4
3	0.50	8.4

#### Reproducibility (Inter-Assay); n = 20

The reproducibility was assessed with 2 stool samples under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [ng/ml]	CV [%]
1	1.84	12.4
2	3.62	6.8

### *Analytical sensitivity*

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB

0.185 ng/ml

### *Accuracy – Trueness*

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, EDN spikes with known concentrations were added to 3 different stool samples.

<b>Sample [ng/ml]</b>	<b>Spike [ng/ml]</b>	<b>Expected [ng/ml]</b>	<b>Obtained [ng/ml]</b>	<b>Recovery [%]</b>
0.28	3.0	3.28	3.79	115.43
	4.0	4.28	4.61	107.64
	5.0	5.28	4.42	83.78
	8.0	8.28	7.39	89.24
0.67	1.5	2.17	2.18	100.41
	2.0	2.67	2.47	92.44
	2.5	3.17	2.96	93.38
	4.0	4.67	4.55	97.41
1.29	0.5	1.79	2.03	112.93
	1.5	2.79	3.22	115.32
	2.0	3.29	3.71	112.72
	3.5	4.79	5.52	115.04

### *Linearity*

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP6-A with a serial dilution of 2 different stool samples.

For EDN in serum plasma, stool and urine, the method has been demonstrated to be linear from 0.42 to 3.99 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than  $\pm 20\%$  in this interval.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
A	1:200	3.359	3.359	100.00
	1:400	1.679	1.736	103.36
	1:800	0.840	0.993	118.26
	1:1600	0.420	0.558	132.99
B	1:200	3.990	3.990	100.00
	1:400	1.995	2.257	113.10
	1:800	0.998	1.156	115.84
	1:1600	0.499	0.547	109.70

### Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to EDN. There was no cross-reactivity observed.

Substance tested	Concentration added [ng/ml]	Obtained [OD]	Conclusion
Lactoferrin	240	0.009	< LoB
Secretory IgA	600	0.008	< LoB
Human albumin	800	0.007	< LoB
PMN elastase	10	0.008	< LoB

## 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 analysed 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 to 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.
- *IDK®* and *IDK Extract®* are trademarks of Immundiagnostik AG.
- 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 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 Immundiagnostik AG along with a written complaint.

## 15. REFERENCES

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.

### Used symbols:

	Temperature limitation		Catalogue Number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		