

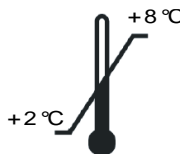
**Manual**

# EDN ELISA Kit

ELISA

***For the determination of EDN (eosinophil derived neurotoxin) in stool***

Valid from 23.11.2022



**IC6500**

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## 1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of EDN (eosinophil derived neurotoxin) in stool. For research use only.

## 2. Introduction

After activation eosinophil granulocytes are segregating the cationic glycoprotein EDN (eosinophil derived neurotoxin). This 18-21 kDa single stranded glycosylated protein is also known as EPX (eosinophil protein X). Together with ECP (eosinophil cationic protein) EDN belongs to the ribonuclease superfamily (1-3). However EDN has a 100-fold increased ribonuclease activity. It is neurotoxic but not cytotoxic (4, 5). The activation of eosinophil granulocytes is important during inflammatory processes in allergic reactions. Thus EDN is a marker for eosinophil activation and degranulation.

The measurement of EDN in stool allows the detection of clinical or subclinical chronic inflammation in the gut. In patients with colitis ulcerosa and/or morbus crohn, the measurement of EDN gives information on the activity of the disease and the prediction of a relapse.

### Applications

- Differentiation of food allergy and food intolerance
- Monitoring of an elimination diet
- Inflammatory processes in the gut
- Intestinal parasitosis

The *ImmuChrom* complete EDN kit allows an easy, rapid and precise quantitative determination of the eosinophil derived neurotoxin in biological samples. The kit includes all reagents ready to use for preparation of the samples.

### **3. General notes, warnings and precautions**

This assay was produced and put on the market for research use only.

Individual components from different batches and test kits should not be interchanged. The expiry dates stated on the relevant packaging must be observed.

The test kit reagents contain preservatives to protect against bacterial growth. Therefore contact with the skin and/or mucous membranes should be avoided.

The test kit contains EDN isolated from human urine from apparently healthy donors. During the purification process, all high-molecular components were separated. As a precautionary measure, all test kit reagents should always be treated as potentially infectious material in accordance with health care accident prevention regulations.

The substrate TMB (tetramethylbenzidine) is toxic by ingestion and skin contact. In the event of contact with the skin, the affected area must be washed immediately with plenty of water and soap.

Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. You should therefore work with protective gloves and goggles. In the event of contact, the burned area must be immediately and thoroughly rinsed with plenty of water. If necessary, a doctor should be consulted.

Adherence to the prescribed protocol for performing the test is essential. ImmuChrom GmbH assumes no liability for any damage caused by unauthorized changes in the test procedure.

The guidelines for carrying out quality control in medical laboratories must be observed. Appropriate controls must be carried along.

The reagents must not be used after the expiration date.

Wear disposable gloves when handling specimens or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or put on makeup in areas where specimens or kit reagents are being handled.

Samples may contain unknown interfering substances. This can lead to false high or false low results.

#### 4. Material delivered in the test package

Article no.	Component	Description	Amount
IC6500mtp	MTP	Microtiter plate coated	12 x 8 wells
IC7400ex	EXT	Extraction buffer	150 ml
IC6500wp	WASHBUF	ELISA waschbuffer conc. 10 fold	100 ml
IC6500st	STD	Standards (1 ml) The concentrations are given in the specification	7 vials
IC6500ko	CTRL	Control 1 and 2 (1 ml) The concentrations are given in the specification	1 vial each
IC6500kg	CONJ	Conjugate, polyclonal peroxidase-labeled antibody	15 ml
IC6000su	SUB	TMB substrate (tetramethylbenzidine)	15 ml
IC6500sp	STOPP	Stop solution	10 ml

#### 5. Additional special equipment

- Centrifuge, 3000xg
- Plastic vials
- Stool sample extraction vials
- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate
- Bidest. water
- ELISA reader with filter 450 nm (reference filter 620 nm)
- Microtiter plate shaker
- Vortex mixer

## 6. Reagent preparation

**Microtiter plate (MTP).** Take the needed number of stripes and assemble them on the holder. Please take care that the plate has reached 20-30 °C before usage. Stripes which are not needed yet must be stored at 2-8 °C. Please do not dispose of the holder until all stripes are used.

**Wash buffer (WASHBUF).** Dilute the wash buffer concentrate 1:10 with bidest. water (1 part buffer + 9 parts bidest. water). The dilution is stable for 14 days at 2-8 °C.

Important: When storing the wash buffer concentrate at 2-8 °C crystallization may occur. Before dilution, all crystals must be dissolved.

It is recommended to dilute only the amount of buffer which is used to process the given samples.

All other test reagents are stable at 2-8 °C up to the date of expiry stated on the label, unless otherwise specified.

## 7. Specimen

### Stool samples

EDN is extracted by the extraction buffer out of the stool sample in a ratio of 1:100 (e.g. 10 mg/ml).

#### Extraction in stick vials

For the extraction stick vials could be used.

We recommend to mix **15 mg** stool with **1.5 ml** EXT, then vortex it until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge it for 10 min at 3000xg.

100 µl of the supernatant are used in the test per well.

## 8. Procedure

### Principle of the method

The EDN-ELISA test determines human EDN according to the “sandwich”-principle. EDN in sample, standard and controls binds to polyclonal antibodies, which are coated to the microtiter plate. After a washing step a peroxidase labeled polyclonal antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm in a microtiter plate reader. The EDN concentration can be calculated from the standard curve.

## Sample preparation

All reagents and samples should be prewarmed to 20-30 °C and mixed well before use.

The position of standards, controls and samples are noted on a protocol sheet.

### 1. **Washing step**

Pick out the pre-assembled microtiter plate with the needed number of stripes and wash them 1x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the washing step.

### 2. **Samples incubation**

Pipette **100 µl STD, CTRL and samples** in double values in the microtiter plate.

The stripes are covered and incubated by shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

### 3. **Washing step**

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

### 4. **Conjugate incubation**

Pipette **100 µl CONJ** in each microwell.

The stripes are covered and incubated by shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

### 5. **Washing step**

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

### 6. **Substrate incubation**

Pipette **100 µl SUB** in each microwell.

Incubate for **10-15 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit) in the dark.

### 7. **Stopping reaction**

Pipette **50 µl STOPP** in each microwell. Mix well.

### 8. **Reading**

Read the absorbance at 450 nm. If the microtiter plate reader allows to use a reference wavelength use 620 nm as reference wavelength.

Reading should be done within 5 min after stopping reaction.

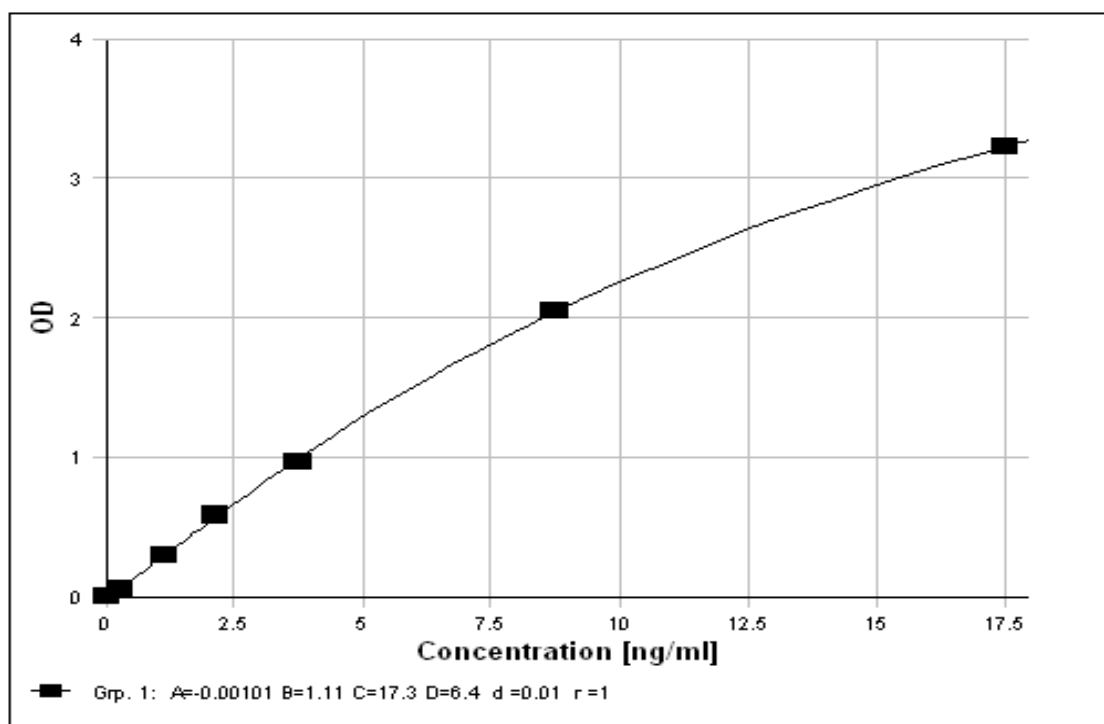
## 9. Calculation of analytical results

For calculating the results we recommend to use the 4-parameter-Marquardt algorithm.

### Stool samples

The obtained EDN concentration is multiplied with 100.

### Standard curve



The curve given above is only for demonstration. It must not be used for calculation of your samples.



## 10. Internal quality control

### Reference values

Stool: < 440 ng/ml

The reference range was established by 2712 samples.

We recommend, that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

## 11. Validation data

### Measuring range

The measuring range of EDN is between a sample concentration of 27.5 - 1750 ng/ml.

### Precision and reproducibility

<b>Intra-Assay CV stool:</b>	8.7 % (1672 ng/ml)	[n = 10]
	6.6 % (730 ng/ml)	[n = 10]
	5.2 % (197 ng/ml)	[n = 10]
<b>Inter-Assay CV stool:</b>	10.1 % (1178 ng/ml)	[n = 10]
	7.8 % (446 ng/ml)	[n = 10]
	8.2 % (218 ng/ml)	[n = 10]

### Detection limit

0.05 ng/ml

For the determination of the detection limit 20 replicates of the standard 0 were measured. After addition of the 3 fold standard deviation to the mean value the concentration was read from the standard curve.

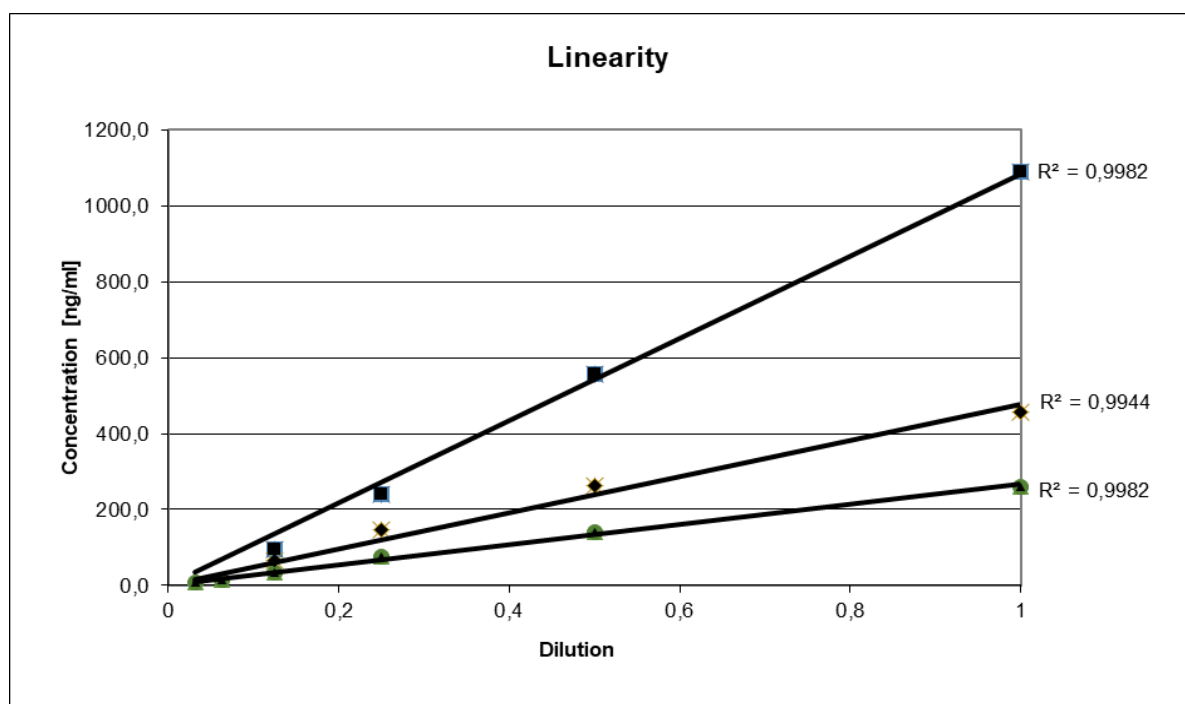
### Limit of quantification

0.1 ng/ml

For the determination of the detection limit 20 replicates of the standard 0 were measured. After addition of the 10 fold standard deviation to the mean value the concentration was read from the standard curve.

## Linearity

Sample	Dilution factor	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	--	--	457	
	1:2	229	263	115
	1:4	114	145	127
	1:8	57.2	65.4	114
2	--	--	1090	
	1:2	545	555	102
	1:4	273	241	88.5
	1:10	109	94.9	87.0
3	--	--	261	
	1:2	131	139	106
	1:4	65.3	76.8	118
	1:8	32.7	34.3	105
	1:16	16.3	13.8	84.5
	1:32	8.2	7.3	89.4



## Recovery

Sample	Endogenous [ng/ml]	Added	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	2.5	4.2	6.6	6.9	105
		12.5	15.0	15.3	102
		25.0	27.5	30.6	111
2	5.2	4.2	9.4	9.0	95.4
		12.5	17.7	17.9	101
		25.0	30.2	26.8	88.7

## 12. Limitations of the method

Stool samples with EDN concentrations above the standard curve should be diluted with extraction buffer (EXT) and measured again.

## 13. Disposal

The substrate (SUB) must be disposed as non-halogenated solvent. The stop solution (STOPP) can be neutralized with NaOH and if the pH value is neutral it can be disposed as salt solution (**important**: this reaction produces heat and should be handled carefully).

Please refer to the appropriate national guidelines.

## 14. Literature references

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