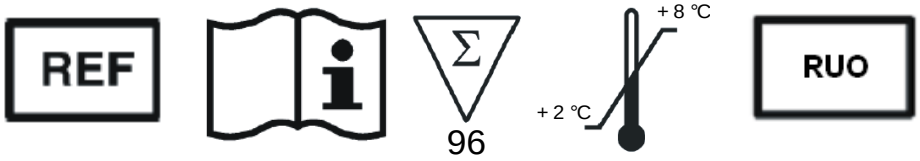
Manual

ß-Defensin 2

ELISA

For the determination of ß-defensin 2 in stool

Valid from 12.11.2021



IC7200

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1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of ß-defensin 2 in stool. For research use only.

2. Introduction

The group of ß-defensins are a class of antimicrobial cationic arginine rich peptides and an integral part of the innate non-specific immune response. So far nine different ß-defensins are known in humans. This includes ß-defensin 2, which consists of 64 amino acids and has a molecular weight of 7 kDa.

An intensified expression of ß-defensins takes place through inflammation and micro-organisms. In Crohn's disease a deficiency of ß-defensin is observed. Due to the restricted barrier function of the intestinal mucosa, the inflammation typical for Crohn's disease can be caused by bacterial invasion.

Applications

- inflammatory processes in the intestine
- integrity of the intestinal mucosa

The ImmuChrom complete ß-defensin 2 kit allows an easy, rapid and precise quantitative determination of ß-defensin 2 in biological samples. The kit includes all reagents ready to use for preparation of the samples.

3. Warnings and precautions

All reagents of this kit are strictly intended for research use only.

Do not interchange kit components from different lots.

The stop solution (STOPP) contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection and appropriate protective clothing in a hood. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapor and avoid inhalation. In case of an accident or indisposition contact a physician immediately.

The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill should be wiped up immediately with copious quantities of water.

Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

Do not pipette by mouth.

Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

The reagents of the test kit contain bactericides to protect against bacterial growth. Avoid the contact with the skin or mucous membrane.

Reagents should not be used beyond the expiration date shown on kit label.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4. Material delivered in the test package

Article no.	Component	Description	Amount
IC7200mtp	MTP	Microtiter plate coated	12 x 8 wells
IC7200wp	WASHBUF	ELISA wash buffer conc. 10-fold	100 ml
IC7400ex	EXT	Extraction buffer	150 ml
IC7200st	STD	Standards (1 ml) (0; 0.1; 0.3; 1.0; 3.0 ng/ml)	5 vials
IC7200ko	CTRL	Controls (2 levels, 1 ml)	1 vial each
IC7200kg	CONJ	Conjugate, peroxidase labeled antibody	15 ml
IC7200vp	SAMPLEBUF	Sample buffer	20 ml
IC7200su	SUB	TMB substrate (tetramethylbenzidine)	15 ml
IC7200sp	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 room temperature 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

The ß-defensin 2 is extracted by the extraction buffer (EXT) out of the stool sample.

Extraction in stick vials

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

The supernatant is diluted **1:2** in sample buffer (e.g. 150 µl supernatant + 150 µl sample buffer).

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

8. Procedure

Principle of the method

The ß-defensin 2-ELISA test determines human ß-defensin 2 according to the “sandwich”-principle. ß-defensin 2 in sample, standard and controls binds to antibodies, which are coated to the microtiter plate. After a washing step a peroxidase labeled detection 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 (against the reference wavelength 620 nm) in a microtiter plate reader. The ß-defensin 2 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. Incubation samples

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

Cover the stripes with a cover film and incubate the microtiter plate by shaking for **60 min** (20-30 °C; 400 rpm, 2 mm orbit diameter).

3. Washing step

Discard the content of the microwells and wash 3x 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.

Cover the stripes with a cover film and incubate the microtiter plate by shaking for **60 min** (20-30 °C; 400 rpm, 2 mm orbit diameter).

5. Washing step

Discard the content of the microwells and wash 3x 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 by shaking for **10-15 min** in the dark (20-30 °C; 400 rpm, 2 mm orbit diameter).

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

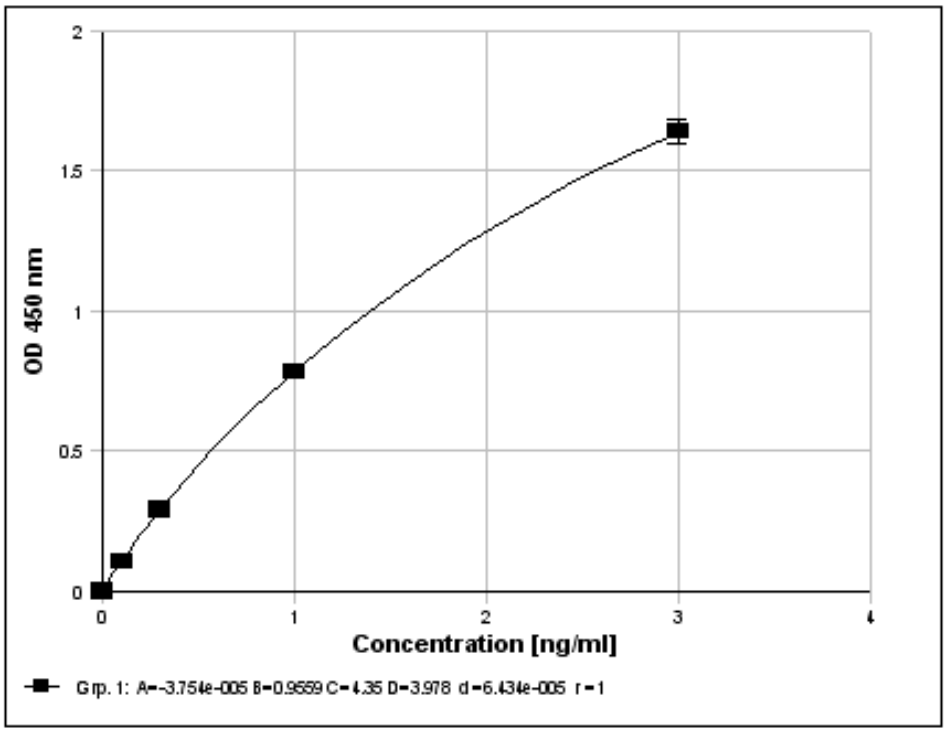
The obtained ß-defensin 2 concentration is multiplied with **200**

Dilution 1: 15 mg in 1.5 ml corresponds to a factor **100** (assumption: 1 g stool = 1 ml)

Dilution 2: Factor **2** (150 µl supernatant + 150 µl sample buffer)

Calculation: Conc. Patient [ng/ml] = obtained conc. [ng/ml] x 100 x 2

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: 8 - 60 ng/ml

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

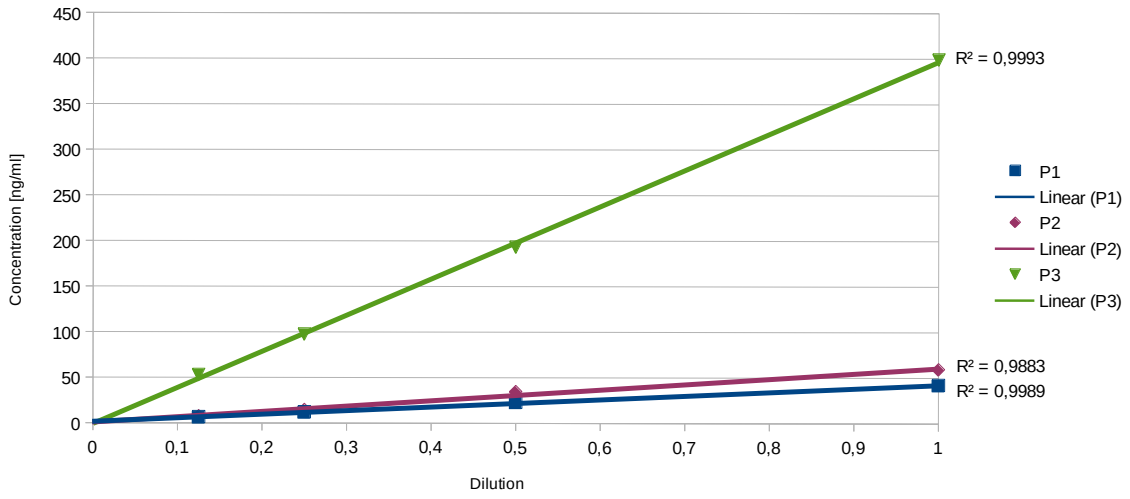
11. Validation data

Precision and reproducibility

Intra-Assay CV:	< 10 % (249 ng/ml)	[n = 10]
	< 10 % (68.6 ng/ml)	[n = 10]
	< 10 % (36.8 ng/ml)	[n = 10]
Inter-Assay CV:	< 15 % (118 ng/ml)	[n = 10]
	< 15 % (67.0 ng/ml)	[n = 10]
	< 15 % (36.7 ng/ml)	[n = 10]

Linearity

The dilution of the samples was performed with sample buffer.



Sample	Dilution	Expected concentration [ng/ml]	Measured concentration [ng/ml]	Recovery [%]
P 1	-	42.4	42.4	-
	1:2	21.2	23.2	109
	1:4	10.6	12.4	117
	1:8	5.30	6.80	128
P 2	-	59.6	59.6	-
	1:2	29.8	34.9	117
	1:4	14.9	14.9	100
	1:8	7.50	7.90	105
P 3	-	399	399	-
	1:2	200	193	96.5
	1:4	99.8	97.7	97.9
	1:8	49.9	53.1	106

Detection limit

Stool 0.009 ng/ml

For the determination the zero-standard was measured 20 times. The 3-fold standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Recovery

Sample	Endogenous [ng/ml]	Added [ng/ml]	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
P 1	11.0	15.0	26.0	26.9	103
		45.0	56.0	59.8	107
		135	146	168	115
P 2	14.0	15.0	29.0	34.6	119
		45.0	59.0	70.5	119
		135	149	140	94.0
P 3	34.8	15.0	49.8	51.6	104
		45.0	79.8	71.0	89.0
		135	170	140	82.4

Cross reactivity

Cross reactivity to other ß-defensins like BD-1, BD-3 and BD-4 could not be detected in stool samples. The used concentration of the substances was 100 ng/ml.

12. Limitations of the method

Stool samples with ß-defensin 2 concentrations above the standard curve should be diluted with sample buffer (SAMPLEBUF) and measured again.

In case of strong diarrhea, it is possible that even patients with an inflammation in the gut show normal values.

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 will produce heat and should be handled carefully).

Please refer to the appropriate national guidelines.

14. Literature references

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