β-Defensin 2 ELISA

For the quantitative determination of Beta-Defensin 2 in human stool

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 31-BD2HU-E01

Size: 96 determinations

Version: 10.10.2025 / ALPCO 1.0

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INTENDED USE

The β-Defensin 2 ELISA is intended for the quantitative determination of beta-defensin 2 in human stool. For Research Use Only. Not for Use in Diagnostic Procedures.

INTRODUCTION

The group of β-defensins are a class of antimicrobial cationic arginine-rich peptides and are part of the innate non-specific immune response. To date, nine different β-defensins have been identified in humans. Defensins are produced by neutrophilic granulocytes and mucosal cells of the small and large intestine.[1] This includes β-defensin 2, which consists of 64 amino acids and has a molecular weight of 7 kDa.

Inflammation and microorganisms result in increased expression of β-defensins.[2] Defensins inhibit the activity of histamine-producing germ species. In addition, they have a modulating effect on the release of histamine from mast cells in the mucous membrane. At low histamine concentrations they cause release, at high concentrations they have an inhibitory effect.[3]

PRINCIPLE OF THE ASSAY

The β-Defensin 2 ELISA test determines human β-Defensin 2 according to the "sandwich" principle. β-Defensin 2 in samples, standards, and controls binds to antibodies coated to the microtiter plate. After a wash step, a peroxidase-labeled detection antibody is added. A second wash 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 of 620 nm) in a microtiter plate reader. The β-Defensin 2 concentration can be calculated from the standard curve.

The test system is calibrated using a reference preparation of recombinant beta-defensin 2 purified from E. coli.

MATERIALS SUPPLIED

31-BD2HU-E01			
Component	**Quantity**	**Preparation**	**Storage**
Microtiter plate, coated	1 plate: 12 x 8-well strips	Ready-to-use	2 - 8˚C
Universal extraction buffer	150 mL	Ready-to-use	2 - 8˚C
ELISA Wash Buffer concentrate, 10X	100 mL	Dilute 1:10	2 - 8˚C
Standards*	5 vials, 1 mL each	Ready-to-use	2 - 8˚C
Control 1 and 2*	2 vials, 1 mL each	Ready-to-use	2 - 8˚C
Conjugate: peroxidase-labeled antibody	15 mL	Ready-to-use	2 - 8˚C
TMB Substrate: tetramethylbenzidine	15 mL	Ready-to-use	2 - 8˚C
Stop solution	10 mL	Ready-to-use	2 - 8˚C
Plate Sealer	2	Ready-to-use	RT

*Please refer to the Certificate of Analysis enclosed with each kit for Standard and Control concentrations.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge, 3000 x g
- Plastic vials
- Stool sample extraction vials (80-EXDEV2-100)
- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate (substrate step)
- Distilled or deionized water
- ELISA reader with 450 nm filter (620 nm reference filter)
- Microtiter plate shaker (2 mm orbital shaker capable of 400 rpm)
- Vortex mixer
- Absorbent paper (lint-free)
- Timer

PRECAUTIONS

- This assay is for Research Use Only. Not for use in diagnostic procedures.
- 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. Contact with the skin and/or mucous membranes should be avoided.
- 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. 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. ALPCO assumes no liability for any damage caused by unauthorized changes to the test procedure.
- Observe guidelines for carrying out quality control in testing laboratories. Appropriate controls must be tested.
- Do not use reagents after the expiration date.
- Wear disposable gloves when handling samples or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or apply makeup in areas where samples or kit reagents are being handled.
- Samples may contain unknown interfering substances. This can lead to false high or false low results.

REAGANT PREPARATION

Microtiter plate. Take the required number of strips and assemble them on the holder. Ensure the plate has reached 20-30°C before use. Strips which are not needed must be stored at 2-8°C in the pouch with desiccant. Please do not dispose of the holder until all strips are used.

Wash buffer. When storing the wash buffer concentrate at 2-8 °C crystallization may occur. Before dilution, all crystals must be dissolved. Dilute the wash buffer concentrate 1:10 with distilled or deionized water (1 part buffer + 9 parts DI water). It is recommended to dilute only the amount of buffer which is used to process the given samples. The 1X working Wash Buffer is stable for 14 days at 2-8°C.

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

SAMPLE PREPARATION

β-Defensin 2 is extracted from stool via a 1:100 dilution with universal extraction buffer before testing. Manual weighing or an approved stool extraction device (80-EXDEV2-100) may be used.

For manual weighing mix **15 mg** stool with **1.5 ml** universal extraction buffer (or greater amount of stool diluted 1:100 with universal extraction buffer), then vortex until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge for 10 min at 3000 x g.

The supernatant (extract) is diluted 1:2 in sample buffer (75 µl supernatant + 75 µl sample buffer). 100 µl of the dilution is used per well in the β-Defensin 2 ELISA.

ASSAY PROCEDURE

All reagents and samples should be equilibrated to 20-30°C and mixed well before use.
The position of standards, controls, and samples are noted on a protocol sheet.

1. **Wash step**

   Take out the needed strips from the microtiter plate and wash 1x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the wash step.

2. **Sample incubation**

   Pipette **100 µl Standards, Controls, and diluted samples** in duplicate in the microtiter plate.

   Cover the strips and incubate with shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

3. **Wash step**

   Discard the contents of the microwells and wash 3x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

4. **Conjugate incubation**

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

Cover and incubate the strips with shaking for **60 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit).

5. **Wash step**

   Discard the content of the microwells and wash 3x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

6. **Substrate incubation**

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

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

7. **Stopping the reaction**

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

8. **Reading**

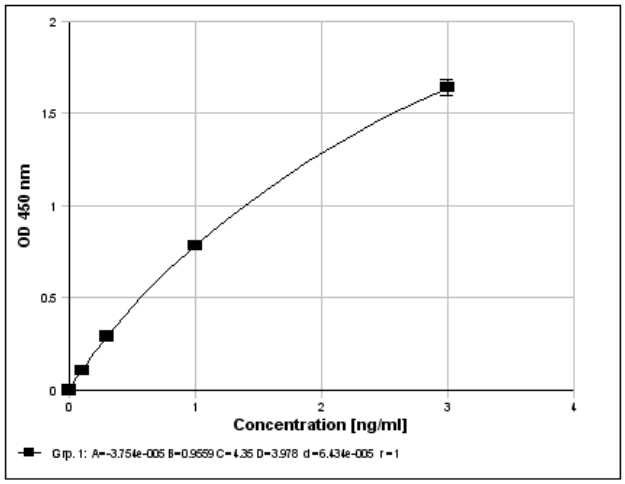
   Read the absorbance at 450 nm. If available, use 620 nm as reference wavelength. Read within **5 minutes** after stopping the reaction.

CALCULATION OF RESULTS

The use of the 4-parameter-Marquardt algorithm is recommended for calculation of results.

For stool samples, the obtained β-Defensin 2 concentration is multiplied by the dilution factor of 200. (1:100 dilution in universal extraction buffer + 1:2 dilution in sample buffer)

TYPICAL STANDARD CURVE



The curve at left is for demonstration purposes only. It must not be used for calculation of sample values.

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range of the β-Defensin 2 ELISA is 4 - 600 ng/ml (following back-calculation for sample dilution).

Sensitivity

Limit of Detection (LOD): 0.01 ng/mL (2 ng/mL following back-calculation for sample dilution)

For the determination of the detection limit, 20 replicates of 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 (LOQ): 0.02 ng/mL (4 ng/mL following back-calculation for sample dilution)

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

Precision: Within run (intra-assay) variation

Intra-Assay CV Stool:	<10 % (249.4 ng/ml)	[n = 10]
	<10 % (68.6 ng/ml)	[n = 10]
	<10 % (36.8 ng/ml)	[n = 10]

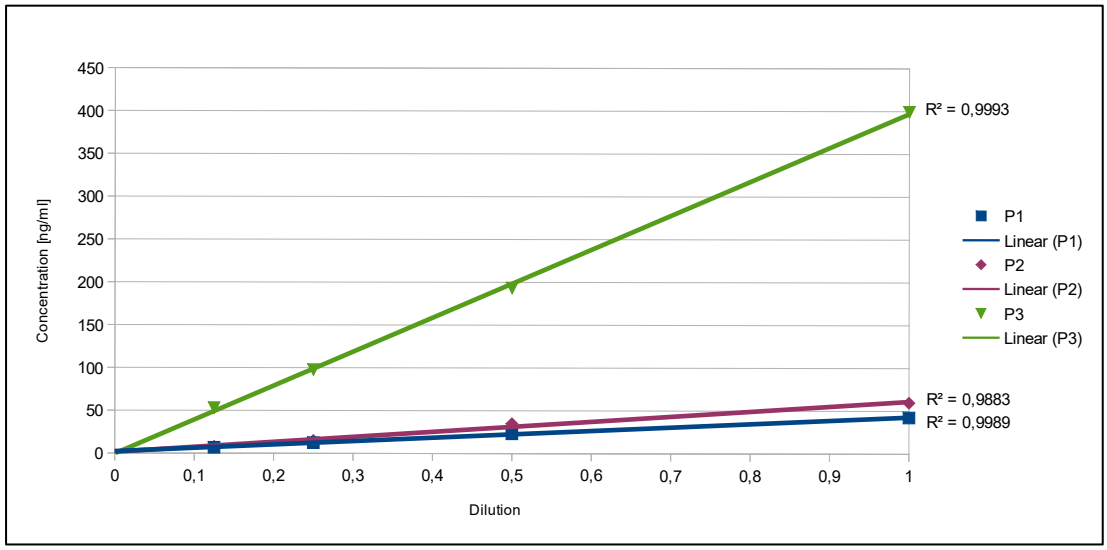
Precision: Between run (inter-assay) variation

Inter-Assay CV stool:	<15 % (117.5 ng/ml)	[n = 10]
	<15 % (67.0 ng/ml)	[n = 10]
	<15 % (36.7 ng/ml)	[n = 10]

Linearity

Note: Sample dilution is performed with sample buffer.

Sample	Dilution Factor	Expected (ng/mL)	Measured (ng/mL)	Recovery (%)
1	--	42.4	42.4	--
	1:2	21.2	23.2	109.2
	1:4	10.6	12.4	116.7
	1:8	5.3	6.8	129.0
2	--	59.6	59.6	--
	1:2	29.8	34.9	117.3
	1:4	14.9	14.9	100.2
	1:8	7.5	7.9	106.2
3	--	399.3	399.3	--
	1:2	199.6	193.3	96.8
	1:4	99.8	97.7	97.8
	1:8	49.9	53.1	106.4



Spike and Recovery

Sample	Endogenous (ng/mL)	Added	Expected (ng/mL)	Measured (ng/mL)	Recovery (%)
1	11.0	15.0 45.0 135.0	26.0 56.0 146.0	26.9 59.8 168.3	103.2 106.7 115.3
2	14.0	15.0 45.0 135.0	29.0 59.0 149.0	34.6 70.5 140.4	119.3 119.4 94.2
3	34.8	15.0 45.0 135.0	49.8 79.8 169.8	51.6 71.0 139.6	103.7 88.9 82.2

Cross-reactivity

Cross reactivity to other β-defensins including BD-1, BD-3, and BD-4 at concentrations of 100 ng/mL could not be detected in stool samples.

Limitations of the Method

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

Disposal

The substrate must be disposed of as non-halogenated solvent. The stop solution can be neutralized with NaOH, and if the pH value is neutral, it can be disposed of as salt solution (**important:** this reaction produces heat and should be handled carefully). Please refer to local and national guidelines.

REFERENCES

1. Siddiqui et al. (2017) *World J Gastrointest Pharmacol Ther* February 6; 8(1): 39-46
2. Pero et al (2017). β-Defensins in the Fight against Helicobacter Pylori. *Molecules* 22, Nr. 3: 424. doi:10.3390/molecules22030424.
3. Kirkamm Hrsg. (2014) Spezielle Labordiagnostik in der naturheilkundlichen Praxis S. 13f. ISBN 978-3-437-56323-2