HUMAN TOTAL β DEFENSIN 103 (BD103) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN TOTAL BD103 CONCENTRATIONS IN SALIVA, EXTRACTED EDTA PLASMA AND CELL CULTURE SUPERNATES.

BD3 IS DETECTABLE IN SALIVA. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN TOTAL BD103 ELISA
Catalog No.	SK00857-06
Lot No.	
Formulation	96 T
Standard range	62 - 4000 pg/mL
Sensitivity	30 pg/mL
Sample Volume	100 µl
Sample Type	Saliva, Extracted EDTA Plasma and Cell Culture Supernates
Dilution factor	2-4 fold dilution for saliva samples. (Optimal dilutions should be determined by each laboratory for each application)
Specificity	Human BD103 full length and mature form
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2 °C-8 °C

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INTRODUCTION

Human Total Beta Defensing 103 immunoassay is a solid phase ELISA designed to measure total human BD103 (full length and mature) in Saliva, cell culture supernates, and EDTA plasma. It contains recombinant human BD103 full length and antibodies raised against this protein. It has been shown to accurately quantify recombinant human BD103 full length and mature form. Results obtained with naturally occurring total BD103 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural total human BD103.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for BD103 full length and mature form has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BD103 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for BD103 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of BD103 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the **SAMPLE SOLUTION** selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate **SAMPLE SOLUTION** and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
BD103 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against BD103.	**857-06-01**	**1 plate**
BD103 Standard – 8 ng /vial of recombinant human BD103 full length in a buffered protein base with preservatives; lyophilized.	**857-06-02**	**1 vial**
Detection Antibody Concentrate – 12 mL/vial, 10-fold concentrated of biotinylated antibody against BD103 with preservatives; lyophilized.	**857-06-03**	**1 vial**
Positive Control - one vial of recombinant human BD103 full length in a buffered protein base with preservatives; lyophilized.	**857-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Sample Solution - 60 mL of solution	**DB30**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservatives	**DB08**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**

TMB Substrate Solution- 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20 °C or – 70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution **(protect from light)** and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 4 months at 2 - 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Saliva – Saliva samples were centrifuged at 10, 000*g* at 4 °C for 20 min, Collect supernatants and were stored at –70 °C until use. Note: 1. Saliva has high concentrations of BD3, wash hand and wear mask to perform standard dilution and or sample dilution as well as assay. 2. Saliva collector must not have any protein binding or filtering capabilities.

Serum Free Cell Culture Supernates - Remove particulates by centrifugation immediately aliquot and store samples at -20 °C ~-70 °C. Avoid repeated freeze-thaw cycles. Cell Culture Supernates require peptide extraction by C18 column as well as other suitable peptide extraction protocol due serum and proteins interface BD103 sample assay. **Note:** Samples that contain serum are not suitable for BD103 assay. Reconstitute lyophilized peptide extractions with 0.22 µm filtered 18.2 mΩ deionized water (without any proteins) in small volume and may require dilution with **Sample Solution** (DB30) to perform assay.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Aliquot and store samples at -20 °C ~-70 °C. Avoid repeated freeze-thaw cycles. EDTA plasma samples require peptide extraction.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

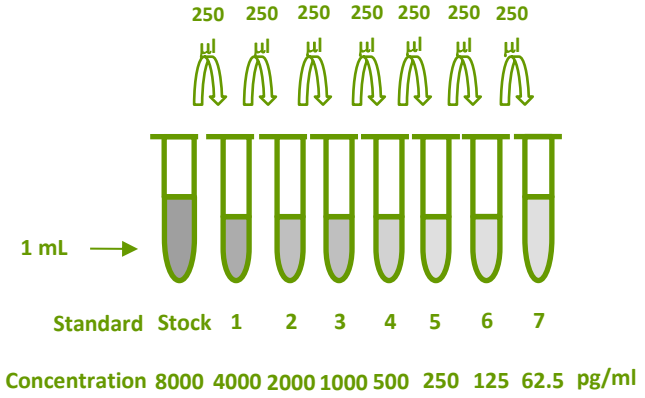
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

BD103 Standard - Refer to vial label for reconstitution volume. Reconstitute the **BD103** standard with 1 mL of **Sample Solution (DB30)**. This reconstitution produces a stock solution of **8000 pg/mL**. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Sample Solution into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **4000 pg/mL** standard serves as the high standard. The Sample Solution serves as the zero standard (0 pg/mL). ***Note:*** *Antibody Diluent Solution (DB08), HRP Diluent Solution (DB01) as well as Wash Buffer (WB01) cannot be used to dilute standard or saliva samples.*

TUBE	STANDARD	SAMPLE SOLUTION	CONCENTRATION
stock	powder	1 mL	8000 pg/ml
# 1	250 µl of stock	250 µl	4000 pg/ml
# 2	250 µl of 1	250 µl	2000 pg/ml
# 3	250 µl of 2	250 µl	1000 pg/ml
# 4	250 µl of 3	250 µl	500 pg/ml
# 5	250 µl of 4	250 µl	250 pg/ml
# 6	250 µl of 5	250 µl	125 pg/ml
# 7	250 µl of 6	250 µl	62.5 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody** with 1.2 mL of **Antibody Diluent Solution (DB08)** to produce a 10-fold concentrated stock solution. Pipette 10.8mL of the appropriate Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. ***Note:*** *1x working solution of Streptavidin-HRP Conjugate should be used within a few days* ***(protect from light)****.*

Positive Control - Reconstitute the positive control with 1.0 mL of **Sample Solution (DB30)** to make Positive Control working solution.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

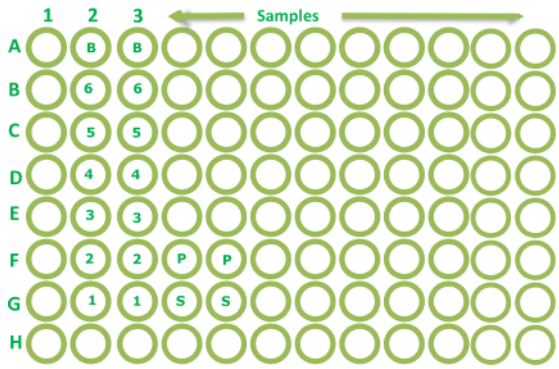
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of **Sample Solution** to Blank wells (A2, A3).
4. Add 100 µL of Standard (from B2, B3 to G2, G3 and G4, G5), samples, or positive control (F4, F5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 25-35 minutes on micro-plate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting

the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the BD103 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human BD103 full length.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of BD103 was 30 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human BD103 full length and mature form.

PROTEINS	CROSSREACTIVITY (%)
Human BD103 full length	100
Human BD103 mature	100
Human BD2	0
Human BD1	0
Human BD4	0
Human BD119	0

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

BD103 (PG/ML)	CORRECTED (450NM)
Blank	0 (0.099)
62.5	0.020
125	0.041
250	0.099
500	0.201
1000	0.442
2000	0.824
4000	1.568

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 25-35 min on plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min