

HUMAN BETA DEFENSIN 1(BD1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN BETA DEFENSIN 1 CONCENTRATIONS
IN SALIVA AND PLASMA



BD1 IS DETECTABLE IN SALIVA. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY. ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN BD1 ELISA KIT
Catalog No.	SK00701-12
Lot No.	
Formulation	96 T
Standard range	7.8 -500 pg/mL
Sensitivity	5 pg/mL
Sample require	100 µL
Dilution Factor	20-40 fold dilution for saliva samples. 50-100 fold dilution for plasma. (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Saliva, Plasma
Specificity	Human BD1
Calibration	Human BD1 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C for 1 month, more information check page 2-3
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Beta Defensin 1 (BD1) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human BD1 from saliva and plasma in a sandwich ELISA format.

This immunoassay contains recombinant Beta Defensin 1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural BD1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human BD1. The capture antibody can bind to the human BD1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human BD1 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human BD1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

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_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
BD1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human BD1.	701-12-01	1 plate
BD1 Standard – refer to lot of recombinant human BD1 in a buffered protein base with preservative; lyophilized.	701-12-02	1 vial
Detection Antibody – refer to lot per vial, 10-fold concentrate of a biotinylated antibody against human BD1 with preservative; lyophilized.	701-12-03	1 vial
Positive Control – one vial of recombinant human BD1; lyophilized.	701-12-04	1 vial
Streptavidin HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin HRP conjugate.	SAHRP	1 vial
Sample Solution - 30 mL of buffered protein based solution with preservative.	DB30	1 bottle
Antibody & HRP Diluent Solution - 25 mL of buffered protein based solution with preservative.	DB08	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 1 month. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate and Antibody & HRP Diluent Solution should be stored at -20° C. Sample Solution, Streptavidin HRP Conjugate and TMB Substrate

Solution should be stored at 2 ~ 8 ° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes. **BD1 IS DETECTABLE IN SALIVA. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY.**

SAMPLE COLLECTION AND STORAGE

Saliva – Collect saliva using a collection device such as a Salivette or equivalent. Saliva samples were centrifuged at 10,000g at 4 °C for 20 min, Collect supernatants and were stored at –70 °C until use.

Note: 1) Saliva has high concentrations of BD1, wash hand and wear mask to perform standard dilution, sample dilution and assay. 2) Saliva collector must not have any protein binding or filtering capability.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) (order Code: 00700-01-25, 25 TIU per vial) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Saliva samples require a 20 or 40-fold dilution. A suggested 20-fold dilution is 12 µL sample + 228 µL

Sample Solution. A suggested 40-fold dilution is 7 µL sample + 273 µL Sample Solution.

EDTA plasma samples require a 50 or 100-fold dilution. A suggested 50-fold dilution is 5 µL sample + 245 µL Sample Solution. A suggested 100-fold dilution is 5 µL sample + 495 µL Sample Solution.

Peptide extraction samples from tissue homogenates or cell culture supernates require lyophilized and **Sample Solution (DB30)** as assay matrix. Reconstitute lyophilized peptide extractions (from Serum, EDTA plasma/Cell Cultures) with 0.22 µm filtered 18.2 mΩ deionized water (without any proteins) in small volume. That should be diluted with **Sample Solution (DB30)** to perform assay

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 1x Wash Buffer.

BD1 Standard - Reconstitute the BD1 standard with **refer to lot** of **Sample Solution (DB30)**. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Sample Solution (DB30) into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **500 pg/mL** standard serves as the high standard. The Sample Solution (DB30) serves as the zero standard (0 pg/mL).

TUBE	STANDARD	Sample Solution (DB30)	CONCENTRATION
stock	powder	Refer to lot	xxxx
# 1	Refer to lot	Refer to lot	500 pg/ml
# 2	250µl of 1	250 µl	250 pg/ml
# 3	250µl of 2	250 µl	125 pg/ml
# 4	250µl of 3	250 µl	62.5 pg/ml
# 5	250µl of 4	250 µl	31.25 pg/ml
# 6	250µl of 5	250 µl	15.6 pg/ml
# 7	250µl of 6	250 µl	7.8 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of **Sample Solution (DB30)** to produce a working solution.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with refer to lot of **Antibody & HRP Diluent Solution (DB08)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody & HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Transfer 120 μL of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 11.88 mL of **Antibody & HRP Diluent Solution (DB08)** to prepare working solution. **(PROTECT FROM LIGHT)**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 μL per well of Sample Solution (DB30) to Blank wells.
3. Add 100 μL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker (300-400 rpm) at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x 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.
5. Add 100 μL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60

minutes on microplate shaker at room temperature. **Protect from light.**

8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well within 3 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

SPECIFICITY









PROTEIN	CROSS-REACTIVITY
Human BD1	100%
Human BD2	0
Human BD3	0

TYPICAL STANDARD CURVE

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (refer to lot)
7.813	0.064
15.625	0.124
31.25	0.232
62.5	0.448
125	0.884
250	1.808
500	2.398

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
 Add 100 µl of standard dilutions, samples, or positive control to the 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 1 hour on the plate shaker at RT. Protect from light.
 Aspirate and wash 4 times.
 Add 100 µl Substrate Solution to each well. Incubate refer to lot on plate shaker at RT. Protect from light.
 Add 100 µl Stop Solution to each well. Read 450nm within 3 min.