HUMAN BETA DEFENSIN 2 (BD2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN BD2 CONCENTRATIONS IN SALIVA, SERUM AND EDTA PLASMA

BD2 IS DETECTABLE IN SALIVA. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY.

PURCHASE INFORMATION:

ELISA NAME	HUMAN BD2 ELISA
Catalog No.	SK00044-01
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	7.8 pg/mL
Sample Volume	100 µl
Sample Type	Saliva, Serum, EDTA Plasma
Dilution Factor	20 for Saliva Samples. 80~160 for Serum. (Optimal dilutions should be determined by each laboratory for each application)
Specificity	Human BD2 only
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2°C - 8°C

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

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INTRODUCTION

Human BD2 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human BD2 in saliva, serum, and EDTA plasma. It contains recombinant human BD2 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human BD2. Results obtained with naturally occurring BD2 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 human BD2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for BD2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BD2 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for BD2 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 BD2 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 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
BD2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against BD2.	**044-01-01**	**1 plate**
BD2 Standard – 1000 pg/vial of recombinant human BD2 in a buffered protein base with preservatives; lyophilized.	**044-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against BD2 with preservatives; lyophilized.	**044-01-03**	**1 vial**
Positive Control - one vial of recombinant human BD2, lyophilized	**044-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Sample Solution – 40 mL of solution with preservatives	**DB30**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Antibody and HRP Diluent Solution - 25 mL of buffered proteins based solution with preservative.	**DB08B**	**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 8 months. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution should be stored at -20 ~ -70°C for up to one month. Diluted standard working solution and positive control should be prepared and used immediately. Streptavidin-HRP Conjugate 100-fold concentrated (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 a desiccant pack and seal along the entire edge of zip-seal. Microplate may be stored for up to 6 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Saliva – Collect saliva using a collection device such as a Salivette or equivalent. Saliva samples were centrifuged at 10,000 x g at 4 °C for 20 min, collect supernatants and were stored at –70 °C until use.
Note: 1. Saliva has high concentrations of BD2, 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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles. Serum samples DO NOT require peptide extraction for BD2 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 DO NOT require peptide extraction for BD2 assay.

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

Saliva samples may require a 20 or 40-fold dilution. A suggested 20-fold dilution is 15 µL of saliva sample + 285 µL Sample Solution. A suggested 40-fold dilution is 10 µL of saliva sample + 390 µL Sample Solution. Any diluted saliva samples should be assayed immediately.
If the samples are lyophilized peptide extractions (from tissue homogenates, cell cultures), reconstitute with 0.22 µm filtered 18.2 mΩ deionized water (without any proteins) in small volume. This should be diluted with Sample Solution to perform BD2 assay.

Serum or EDTA **Plasma** samples may require an 80 or 160-fold dilution. A suggested 80-fold dilution is 5 µL sample + 395 µL Sample Solution. A suggested 160-fold dilution is 5 µL sample + 395 µL Sample Solution, following 150 µl of 80-fold diluted sample + 150 µl of Sample Solution.

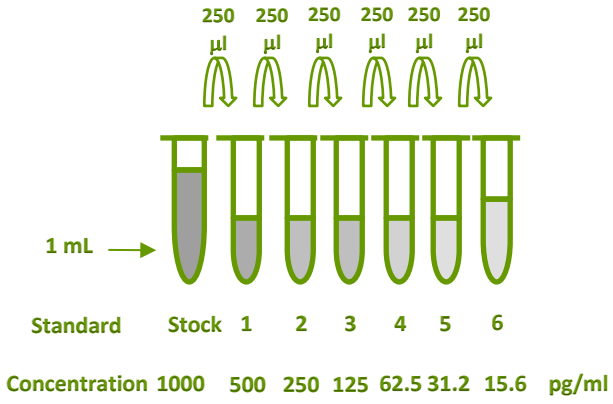
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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.
BD2 Standard - Refer to vial label for reconstitution volume. Reconstitute the **BD2** Standard with 1 mL of **Sample Solution (DB30)**. This reconstitution

produces a stock solution of 1000 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 #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The Sample Solution serves as the zero standard (0 pg/mL).

TUBE	STANDARD	SAMPLE SOLUTION	CONCENTRATION
Stock	Powder	1 mL	1000 pg/ml
# 1	250 µl of stock	250 µl	500 pg/ml
# 2	250 µl of 1	250 µl	250 pg/ml
# 3	250 µl of 2	250 µl	125 pg/ml
# 4	250 µl of 3	250 µl	62.5 pg/ml
# 5	250 µl of 4	250 µl	31.25 pg/ml
# 6	250 µl of 5	250 µl	15.6 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody and HRP Diluent Solution (DB06)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody and HRP Diluent Solution (DB06)** into a 15 ml centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare 1x working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **Antibody and HRP Diluent Solution (DB08B)** 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.

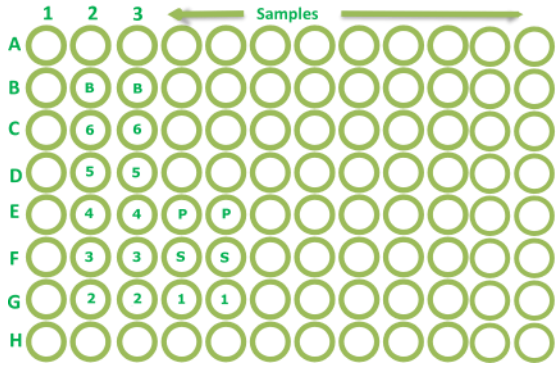
Positive Control - Reconstitute the Positive Control stock with 0.5 mL of **Sample Solution (DB30)** to prepare Positive Control working solution. **Note:** Positive control should be prepared and used immediately.

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 duplicates.

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 (B2, B3).
4. Add 100 µL of Standard (from C2, C3 to G2, G3 and G4, G5 to F4, F5), Sample, or Positive Control (E4, E5) 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 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.
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 6-10 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 BD2 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.

TYPICAL DATA

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

STANDARD (PG/ML)	CORRECTED (450NM)
BLANK	0 (0.126)
15.6	0.061
31.25	0.124
62.5	0.232
125	0.419
250	0.549
500	0.700
1000	1.066

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human BD2.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of BD2 was 7.8 pg/mL.

LINEARITY

To assess the linearity of the assay, pooled research human saliva samples were diluted with Sample Solution (DB30) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
20 X	442.31	8846.20	100
40 X	236.150	9446.00	107

To assess the linearity of the assay, pooled research human serum samples were diluted with Sample Solution (DB30) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
80 X	606.91	48552.8	100
160 X	360.14	57622.4	118

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with Sample Solution (DB30) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
20 X	724.25	14485.0	100
40 X	340.88	13635.2	94
80 X	207.74	16619.2	115

SPECIFICITY

This assay recognizes both natural and recombinant human BD2. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh BD2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Human Recombinant Proteins:

BD1
BD3

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 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 6-10 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min