HUMAN BETA DEFENSIN-1 (BD-1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN BD-1 CONCENTRATIONS IN SALIVA, PLASMA AND SERUM

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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:

ELISA NAME	**HUMAN BETA DEFENSIN-1 (BD-1) ELISA**
Catalog No.	SK00701-06
Formulation	96 T
Standard range	156 - 10000 pg/mL
Sensitivity	78 pg/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, Plasma, Saliva
Specificity	Human BD-1
Calibration	Human BD-1 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

THIS IS ONE TIME USE ONLY!

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DESCRIPTION

This Human Beta Defensin-1 (BD-1) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human BD-1 from saliva, serum and plasma in a sandwich ELISA format.
This immunoassay contains recombinant human BD-1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural BD-1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human BD-1. The capture antibody can bind to the human BD-1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human BD-1 is added to the wells. After another washing of the plate, the streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human BD-1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
BD-1 Microplate – 96 well microplate coated with monoclonal antibody specific for human BD-1.	**701-06-01**	**1 plate**
BD-1 Standard – refer to lot specific vial of lyophilized recombinant human BD-1	**701-06-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against human BD-1.	**701-06-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant BD-1	**701-06-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered solution with preservative.	**DB06**	**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**

PROCEDURAL LIMITATIONS

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

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.
This ELISA Kit is one time use only. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8 °C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8 °C for up to 8 months.

Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 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.

SAMPLE COLLECTION AND STORAGE

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.
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.

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 with a pretest. Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer. If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved.

BD-1 Standard – Reconstitute the human BD-1 standard with refer to lot specific of Dilution Buffer. The concentration of the reconstituted stock solution is refer to lot specific. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Refer to lot specific	10 ng/mL
# 1	250 µL of stock	250µL	5 ng/mL
# 2	250µL of 1	250µL	2.5 ng/mL
# 3	250µL of 2	250µL	1.25 ng/mL
# 4	250µL of 3	250µL	0.625 ng/mL
# 5	250µL of 4	250µL	0.312 ng/mL
# 6	250µL of 5	250µL	0.156 ng/mL

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 10.8 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 10.8 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **(protect from light)**. **DO NOT FREEZE.**

Positive Control - Reconstitute the Positive Control with 1.0 mL Dilution Buffer. **Note:** Positive Control could be used within a few days if stored at -20 °C or -70 °C.

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, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells (A2, A3).
4. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution (B2, B3 to G2, G3), **sample,** or **positive control** (C4, C5). Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature. (Please see plate layout provided.)
5. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 90 minutes on microplate shaker at room temperature.
7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of working solution of **Streptavidin HRP conjugate**. Cover with plate sealer and incubate for 40 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration and wash as in step 5.
10. Add 100 µL per well of **Substrate Solution**. Incubate for 10-15 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Read plate using a microplate reader set to 450 nm within 5 minutes.

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 BD-1	100%
Human BD-3	0
Human BD-2	0

TYPICAL STANDARD CURVE

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.122)
0.156	0.023
0.312	0.041
0.625	0.081
1.25	0.189
2.5	0.467
5	1.051
10	2.603

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
Add 100 µL of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
Aspirate and wash 4 times.
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 90 minutes on microplate shaker at RT.
Aspirate and wash 4 times.
Add 100 µL per well of Streptavidin-HRP Conjugate working solution. Cover with plate sealer and incubate 40 minutes on microplate shaker at RT. **Protect from light.**
Aspirate and wash 4 times.
Add 100 µL per well of Substrate Solution. Incubate 15-20 min on microplate shaker at RT. **Protect from light.**
Add 100 µL per well of Stop Solution. Read at 450 nm within 5 minutes.