

HUMAN BETA DEFENSIN-4A (BD-4A) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN BD-4A CONCENTRATIONS IN SALIVA,
PLASMA AND SERUM



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 BETA DEFENSIN-4A (BD-4A) ELISA
Catalog No.	SK00856-06
Formulation	96 T
Standard range	15.6 - 1000 pg/mL
Sensitivity	7 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-4A full and mature
Calibration	Human BD-4A Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C for 1 month. More information check page 2
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-4A (BD-4A) /BD2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human BD-4A from saliva, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human BD-4A and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural BD-4A samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human BD-4A. The capture antibody can bind to the human BD-4A in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human BD-4A 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-4A 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

_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.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
BD-4A Microplate – 96 well microplate coated with monoclonal antibody specific for human BD-4A.	856-06-01	1 plate
BD-4A Standard – refer to lot specific vial of lyophilized recombinant human BD-4A	856-06-02	1 vial
Detection Antibody Concentrate – refer to lot of 10-fold concentrate of lyophilized biotinylated antibody against human BD-4A.	856-06-03	1 vial
Positive Control – one vial of lyophilized recombinant BD-4A	856-06-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	SAHRP	1 vial
Dilution Buffer – 40 mL of buffered solution with preservative.	DB30	1 bottle
Antibody & HRP Diluent Solution – 25 mL of buffered 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, Dilution Buffer and Antibody & HRP diluent Solution should be stored at -20 °C. Streptavidin-HRP Conjugate concentrated 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 (300 – 450 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 $\leq -20^{\circ}\text{C}$. 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 $\leq -20^{\circ}\text{C}$. 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-4A Standard – Reconstitute the human BD-4A 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	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 - Reconstitute the Detection Antibody Concentrate with refer to lot of **Antibody & HRP Diluent Solution (DB08)** to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. 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 - Pipette 11.88 mL of **Antibody & HRP Diluent Solution (DB08)** 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.

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 μ L per well of **Dilution Buffer** to Blank wells (A2, A3).
4. Add 100 μ L 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 overnight at 2-8 °C.
5. Aspirate and wash each well with 300 μ L 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 μ L per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration and wash as in step 5.
8. Add 100 μ L per well of working solution of **Streptavidin HRP conjugate**. Cover with plate sealer and incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration and wash as in step 5.
10. Add 100 μ L per well of **Substrate Solution**. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
11. Add 100 μ L 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-4A	100%
Human BD-3	0
Human BD-1	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 (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.102)
15.6	0.039
31.25	0.062
62.5	0.112
125	0.219
250	0.467
500	1.151
1000	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 overnight at 2-8 °C.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 2 hours 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 60 minutes on microplate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate refer to lot on microplate shaker at RT. Protect from light.
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 5 minutes.

SAMPLE TEST

The research samples were diluted by Dilution Buffer (DB30) and assayed by Beta Defensin-4A (Human) ELISA Kit SK00856-06

SAMPLE TYPE	DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
Human Saliva	20 X	472.920	9.458	100
Human Saliva	40 X	230.451	9.218	97.5
Human Serum	80 X	764.283	61.142	100
Human Serum	160 X	380.419	61.867	101
Human Plasma	25 X	627.103	15.678	100
Human Plasma	50 X	307.623	15.381	98.1
Human Plasma	100 X	143.247	14.324	91.4