HUMAN DKK-3 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN DKK-3 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

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.

PURCHASE INFORMATION:
THIS IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN DKK-3 ELISA KIT**
Catalog No.	SK00312-09
Lot No.	
Formulation	96 T
Standard range	15.6 - 1000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Dilution Factor	80 **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human DKK-3 only
Calibration	Human DKK-3 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.	

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INTRODUCTION

Human DKK-3 immunoassay is a solid phase ELISA designed to measure human DKK-3 in cell culture supernates, serum and plasma. It contains recombinant human DKK-3 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human DKK-3. Results obtained with naturally occurring DKK-3 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 DKK-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for DKK-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any DKK-3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for DKK-3 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 DKK-3 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 Dilution Buffer 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 Dilution Buffer 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
DKK-3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against DKK-3.	**312-02-01**	**1 plate**
DKK-3 Standard – 1000 pg/vial of recombinant human DKK-3 in a buffered protein base with preservative; lyophilized.	**312-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/ vial, 10-fold concentrate of biotinylated antibody against DKK-3 with preservative; lyophilized.	**312-02-03**	**1 vial**
Positive Control – one vial of recombinant human DKK-3; lyophilized.	**312-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody & HRP Diluent Solution – 30 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 for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C or -70° C.

Streptavidin-HRP Conjugate and **TMB Substrate Solution** should be stored only at 2~ 8 °C. Do not use kit past expiration date.

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 be 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 case of contact with skin or eyes wash immediately with water.

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

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 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) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require an 80-fold dilution or greater. A suggested 80-fold dilution is 3 µL sample + 237 µL Dilution Buffer. **However, optimal dilution should be determined by each laboratory for each application.**
Use polypropylene test tubes.

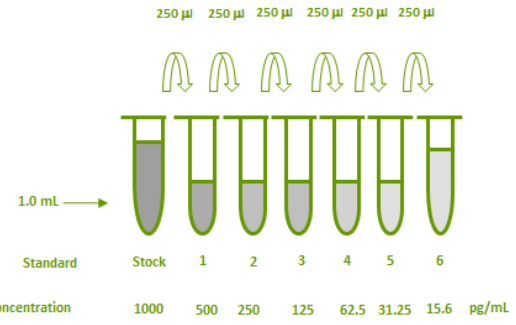
REAGENT PREPARATION

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.

DKK-3 Standard - Reconstitute the DKK-3 standard with 1.0 mL of Dilution Buffer. 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 Dilution Buffer 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 Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	powder	1.0 ml	1000 pg/ml
# 1	250µl of stock	250µl	500 pg/ml
# 2	250µl of 1	250µl	250 pg/ml
# 3	250µl of 2	250µl	125 pg/ml
# 4	250µl of 3	250µl	62.5 pg/ml
# 5	250µl of 4	250µl	31.25 pg/ml
# 6	250µl of 5	250µl	15.625 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL 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 - 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. **Note:** 1x working solution of Streptavidin-HRP conjugate should be freshly prepared and used within a few hours **(protect from light).**

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 microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions in reverse order of serial dilutions #6 to #S, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 microplate 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 microplate 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 3-7 minutes on microplate 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 3 minutes, using a microplate 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 DKK-3 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

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

DKK-3 Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.092)
15.625	0.049
31.25	0.103
62.5	0.205
125	0.382
250	0.685
500	1.280
1000	2.082

SPECIFICITY

No significant cross-reactivity or interference was observed.

PROTEINS	CROSSREACTIVITY (%)
Human DKK-3	100
Mouse DKK-3	0
Human DKK-1	0

LINEARITY

To assess the linearity of the assay pooled research human **serum** samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
40x	671.323	26.853	100
80x	366.019	29.282	109

To assess the linearity of the assay pooled research human **EDTA plasma** samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
40x	529.572	21.183	100
80x	302.171	24.174	114

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard dilutions, samples, or 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 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 3-7 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 3 min.