DKK4 (Human) ELISA Kit

Catalog Number KA1707

96 assays

Version: 02

Intended for research use only

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Introduction

Principle of the Assay

DKK4 (Human) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Dkk-4 in serum, plasma, cell culture supernatants. This assay employs an antibody specific for human Dkk-4 coated on a 96-well plate. Standards and samples are pipetted into the wells and Dkk-4 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Dkk-4 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Dkk-4 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

General Information

Materials Supplied

Component	Amount
Dkk-4 Microplate (Item A): 96 wells coated with anti-human Dkk-4.	96 (8 x 12) wells
Wash Buffer Concentrate (20x) (Item B): 20x concentrated solution	25 mL
Standard Protein (Item C): Human Dkk-4. 1 vial is enough to rum each standard in duplicate.	2 vials
Assay Diluent (Item E): 5x concentrated buffer.	15 mL
Detection Antibody Dkk-4 (Item F): Biotinylated anti-human Dkk-4 (each vial is enough to assay half microplate).	2 vials
HRP-Streptavidin concentrates (Item G): 200x concentrated HRP-conjugated Streptavidin.	200 µL
TMB One-Step Substrate Reagent (Item H): 3, 3', 5, 5'-tetramethylbenzidine (TMB) in buffered solution.	12 mL
Stop Solution (Item I): 0.2 M sulfuric acid.	8 mL

Storage Instruction

May be stored for up to 6 months at 2 to 8°C from the date of shipment. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Reconstituted standard can be stored at -80°C for up to 1 week.
Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Precision pipettes to deliver 2 µL to 1 mL volumes.
- ✓ Adjustable 1-25 mL pipettes for reagent preparation.
- ✓ 100 mL and 1 liter graduated cylinders.
- ✓ Absorbent paper.
- ✓ Distilled or deionized water.
- ✓ Log-log graph paper or computer and software for ELISA data analysis.
- ✓ Tubes to prepare standard or sample dilutions.

Assay Protocol

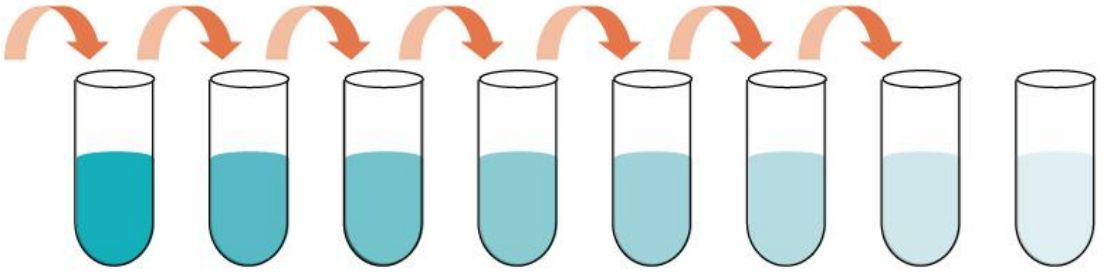
Reagent Preparation

- ✓ Bring all reagents and samples to room temperature (18 - 25°C) before use.

- ✓ Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.

- ✓ Preparation of standard: Briefly spin the vial of Item C. Add 400 µL 1X Assay Diluent A (Item E) into Item C vial to prepare a 50 ng/mL standard solution. Dissolve the powder thoroughly by a gentle mix. Add 100 µL Dkk-4 standard from the vial of Item C, into a tube with 400 µL 1X Assay Diluent to prepare a 10,000 pg/mL standard solution. Pipette 400 µL 1X Assay Diluent into each tube. Use the 10,000 pg/mL standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent serves as the zero standard (0 pg/mL).

100 µL standard
+ 400 µL 200 µL 200 µL 200 µL 200 µL 200 µL 200 µL



10,000 pg/mL | 3333 pg/mL | 1111 pg/mL | 370.4 pg/mL | 123.5 pg/mL | 41.15 pg/mL | 13.72 pg/mL | 0 pg/mL

- ✓ If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

- ✓ Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1X Assay Diluent (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent (Item E) and used in step 4 of Assay Procedure.

- ✓ Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent (Item E).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 50 µL of HRP-Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent to prepare a final 200 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Sample Preparation

✓ Sample dilution: 1X Assay Diluent (Item E) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold.

Note: Levels of Dkk-4 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µL of each standard (see Reagent Preparation) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette 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.
4. Add 100 µL of 1X prepared biotinylated antibody (see Reagent Preparation) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

✓ Summary

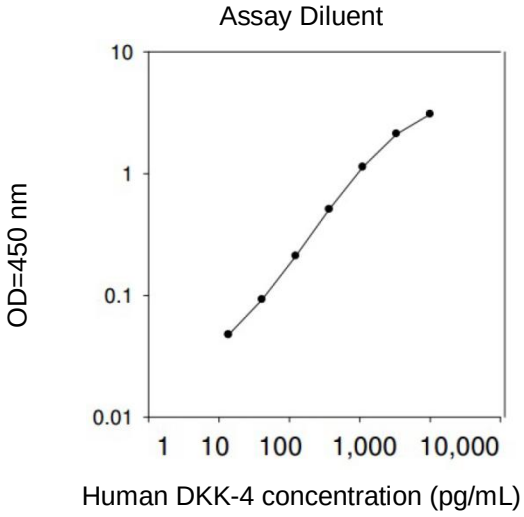
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.
3. Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

These standard curves are for demonstration only. A standard curve must be run with each assay.



Performance Characteristics

- Sensitivity

The minimum detectable dose of Human Dkk-4 alpha is was determined to be 10 pg/mL.
Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

- Spiking & Recovery

Recovery was determined by spiking various levels of Human Dkk-4 into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	95.59	85-105
Plasma	82.62	69-91
Cell culture media	109.6	97-121

- Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	117.7	124.7	122.2
	Range (%)	106-125	117-129	114-129
1:4	Average % of Expected	118.6	118.0	120.7
	Range (%)	107-127	109-126	108-127

- Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

- Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, MMP-1, -2, -3, -10, PARC, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.

Resources

Troubleshooting

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. Check pipettes 2. Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing
2. Low signal	1. Improper preparation of standard and/or biotinylated antibody 2. Too brief incubation times 3. Inadequate reagent volumes or improper dilution	1. Briefly spin down vials before opening. Dissolve the powder thoroughly 2. Ensure sufficient incubation time; assay procedure step 2 may be done overnight 3. Check pipettes and ensure correct preparation
4. Large CV	1. Inaccurate pipetting 2. Air bubbles in wells	1. Check pipettes 2. Removed bubbles in wells
5. High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed 2. Make fresh wash buffer
6. Low sensitivity	1. Improper storage of the ELISA kit 2. Stop solution	1. Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light 2. Add stop solution to each well before reading plate

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H