Human Dkk3 / REIC ELISA Pair Set

(Dickkopf related protein 3)

Catalog Number : SEK10203

To achieve the best assay results, this manual must be read carefully before using this product and the assay is run as summarized in the General ELISA protocol.

BACKGROUND

Dkk3, also known as REIC (Reduced Expansion in Immortalized Cells), is a member of the dickkopf-related family which has distinct patterns of expression in adult and embryonic tissues. The Dickkopf (Dkk) family is composed of four main members (Dkk1 , Dkk2 , Dkk3 , Dkk4), which typically regulate Wnt/beta-catenin signaling. An exception is Dkk3, which does not affect Wnt/beta-catenin signaling. Dkk3 is a secreted protein with two cysteine-rich domains separated by a linker region, and has a wide range of effects on tissue development and morphogenesis through its interactions with the Wnt signaling pathway. The expression of Dkk3 gene is down regulated in human immortalized cells and a variety of tumors, such as hepatoma, lung carcinomas and malignant melanoma. Meanwhile, overexpression of Dkk3 results in tumor cell line-specific growth inhibition and apoptosis. Dkk3 may function as a suppressor gene of tumor growth and be regarded as a promising potential therapeutic target.

Dickkopf (Dkk)-3, a member of Dkk family of Wnt antagonists, is frequently inactivated in lung cancer and plays a role in suppressing lung cancer cell growth through inhibition of beta-catenin/T-cell factor (TCF)-4 signaling. Dkk3 is the only Dkk family member abundantly expressed in normal lung, but silenced by promoter hypermethylation in a large fraction of lung cancer cell lines and lung tumors. Downregulation of Dkk3 was correlated with tumor progression and expression of nuclear beta-catenin in lung tumors. Ectopic expression of Dkk3 in lung cancer cells with Dkk3 hypermethylation induced apoptosis and inhibited TCF-4 activity as well as nuclear accumulation of beta-catenin and expression of TCF-4 targets c-Myc and cyclin D1. Epigenetic inactivation of Dkk3 activates the Wnt/beta-catenin pathway, thereby promoting the growth of lung cancer cells. Dkk3 methylation also proves to be a novel prognostic marker potentially useful in the clinical management of human breast cancer.

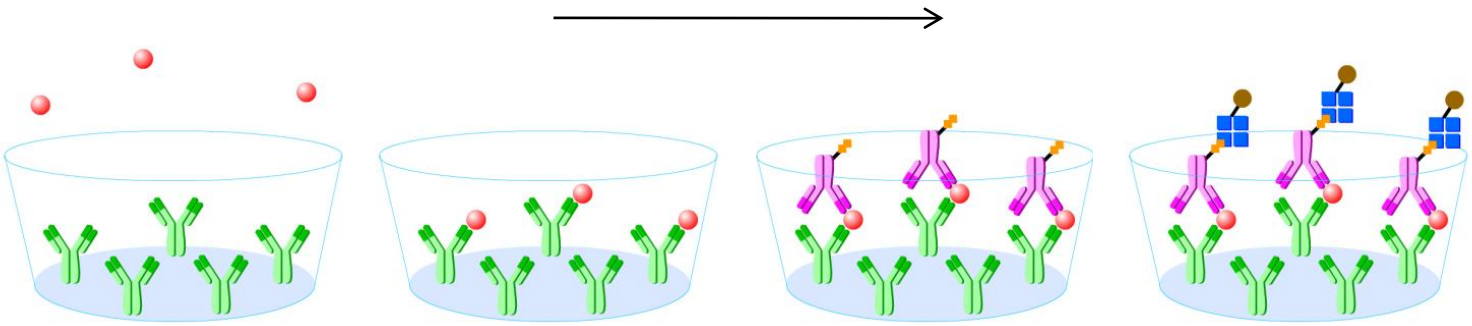
PRINCIPLE OF THE TEST

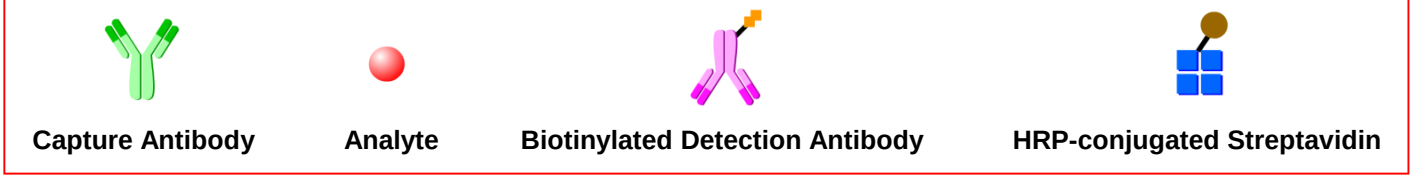
The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Dkk3 / REIC coated on a 96-well plate. Standards and samples are added to the wells, and any Dkk3 / REIC present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti- Dkk3 polyclonal antibody is then added, producing an antibody-antigen-antibody “sandwich”. To produces color in proportion to the amount of Dkk3 / REIC present in the sample strepavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450 nm.

INTENDED USE

- The human Dkk3 / REIC ELISA Pair Set is for the quantitative determination of human Dkk3.
- This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY





This Pair Set has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody - 0.5 mg/mL of mouse anti-DKK3 monoclonal antibody. Dilute to a working concentration of 2 µg/mL in CBS before coating. (Catalog: # 10203-MM05T)

Detection Antibody - Each vial contains 120 µg biotinylated rabbit anti-DKK3 polyclonal antibody. Reconstitute with sterile 1 mL distilled water. Dilute to a working concentration of 1 µg/mL in detection antibody dilution buffer before use

Standard - Each vial contains 98 ng of recombinant DKK3. Reconstitute with 1 mL detection antibody dilution buffer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 4000 pg/mL is recommended

Streptavidin-HRP - 50 µL of streptavidin conjugated to horseradish-peroxidase. 1:2000 Dilution in detection antibody dilution buffer before use

SOLUTIONS REQUIRED

CBS - 0.05M Na_2CO_3 , 0.05M $NaHCO_3$, pH 9.6, 0.2 µm filtered

TBS - 25mM Tris, adjust pH to 7.4 by HCl

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered.

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na_2HPO_4 and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2 , mix it well

Stop Solution - 2 N H_2SO_4

PRECAUTION

The Stop Solution suggested for use with this Pair Set is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Standard: Store lyophilized standard at -20℃ to -80℃ for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -20℃ to -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

Streptavidin-HRP: Store at 4℃ and protect it from prolonged exposure to light. **DO NOT FREEZE!** It is stable for up to 6 months from date of receipt.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100μL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300μl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

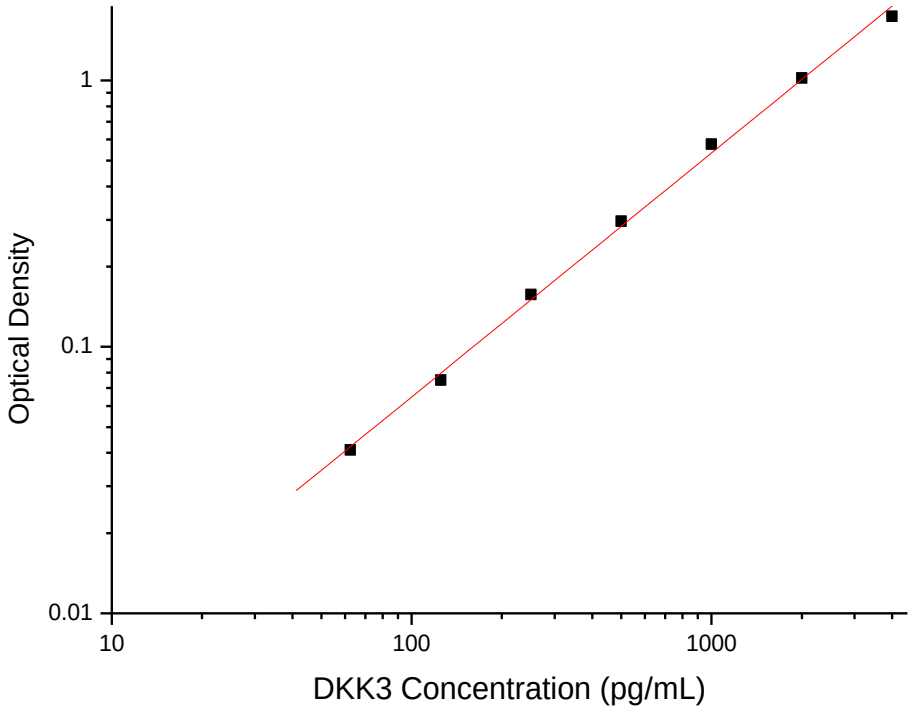
1. Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100 μL of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. Add 200 μL of substrate solution to each well. Incubate for 20 minutes at room temperature **(if substrate solution is not as requested, the incubation time should be optimized)**. Avoid placing the plate in direct light.
8. Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- 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.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
62.5	0.041
125	0.075
250	0.157
500	0.296
1000	0.577
2000	1.019
4000	1.741

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of human Dkk3 / REIC was determined to be approximately **62.5 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

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

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Notes