Sino Biological
Biological Solution Specialist

Human CD3d / CD3 delta ELISA Pair Set

Catalog Number : SEK10981

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

T-cell surface glycoprotein CD3 delta chain, also known as CD3D, is a single-pass type I membrane protein. CD3D, together with CD3-gamma, CD3-epsilon and CD3-zeta, and the T-cell receptor alpha/beta and gamma/delta heterodimers, forms the T cell receptor-CD3 complex. The majority of T cell receptor (TCR) complexes in mice and humans consist of a heterodimer of polymorphic TCRalpha and beta chains along with invariant CD3gamma, delta, epsilon, and zeta chains. CD3 chains are present as CD3gammaepsilon, deltaepsilon, and zetazeta dimers in the receptor complex and play critical roles in the antigen receptor assembly, transport to the cell surface, and the receptor-mediated signal transduction. T cell receptor-CD3 complex plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways. This complex is critical for T-cell development and function, and represents one of the most complex transmembrane receptors. The T cell receptor-CD3 complex is unique in having ten cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs). CD3D contains 1 ITAM domain and has been shown to interact with CD8A. In the mouse, knockout of CD3delta allows some degree of T lymphocyte differentiation since mature CD4 and CD8 as well as TCRgammadelta T lymphocytes are observed in the periphery. In contrast, deleterious mutation of the CD3delta encoding gene in the human leads to a severe combined immunodeficiency characterised by the complete absence of mature T cell subpopulations including TCRalpha/beta and TCRgamma/delta. Defects in CD3D cause severe combined immunodeficiency autosomal recessive T-cell-negative/B-cell-positive/NK-cell-positive (T-/B+/NK+ SCID) which is a genetically and clinically heterogeneous group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. In humans the absence of CD3 delta results in a complete arrest in thymocyte development at the stage of double negative to double positive transition and the development of gamma delta T-cell receptor-positive T cells is also impaired.

PRINCIPLE OF THE TEST

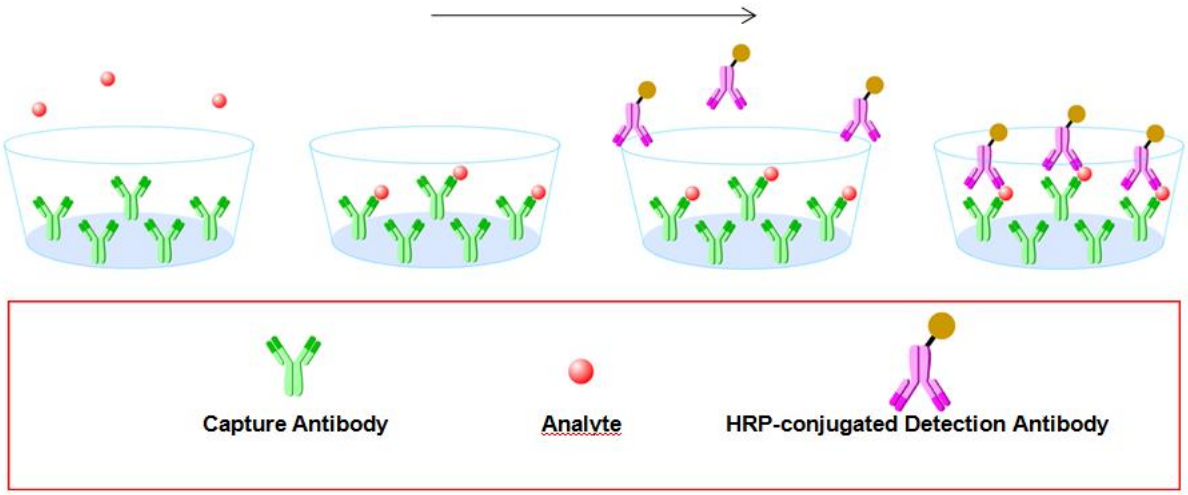
The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for CD3d / CD3 delta coated on a 96-well plate. Standards and samples are added to the wells,and any CD3d / CD3 delta present binds to the immobilized antibody. The wells arewashed and a horseradish peroxidase conjugated mouse anti-TrkB monoclonalantibody is then added, producing an antibody-antigen-antibody "sandwich". Thewells are again washed and TMB substrate solution is loaded, which producescolor in proportion to the amount of CD3d / CD3 delta present in the sample. Toend the enzyme reaction, the stop solution is added and absorbances of themicrowell are read at 450 nm.

INTENDED USE

◆ The human CD3D ELISA Pair Set is for the quantitative determination of human CD3D .

◆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 – 0.3 mg/mL of mouse anti-CD3D monoclonal antibody. Dilute to a working concentration of 1 µg/mL in CBS before coating (Catalog: # 10981-MM07)

Detection Antibody – 0.5 mg/mL mouse anti-CD3D monoclonal antibody conjugated to horseradish-peroxidase (HRP). Dilute to working concentration of 0.25 µg/mL in detection antibody dilution buffer before use. (Catalog: # 10981-MM08)

Standard – Each vial contains 60 ng of recombinant CD3D. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. A seven-point standard curve usi ng 2-fold serial dilutions in sample dilution buffer, and a high standard of 4 ng/mL is recommended.
Standard reconstitution tips: Add dilution buffer, gently mix it up and down 3~5 times. Avoid violent and long-time shock.

SOLUTIONS REQUIRED

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

TBS - 20 mM Tris, 150 mM NaCl, pH 7.4

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 250 µl 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: Protect it from prolonged exposure to light. Aliquot and store at -20℃ to -80℃ and 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 -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

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

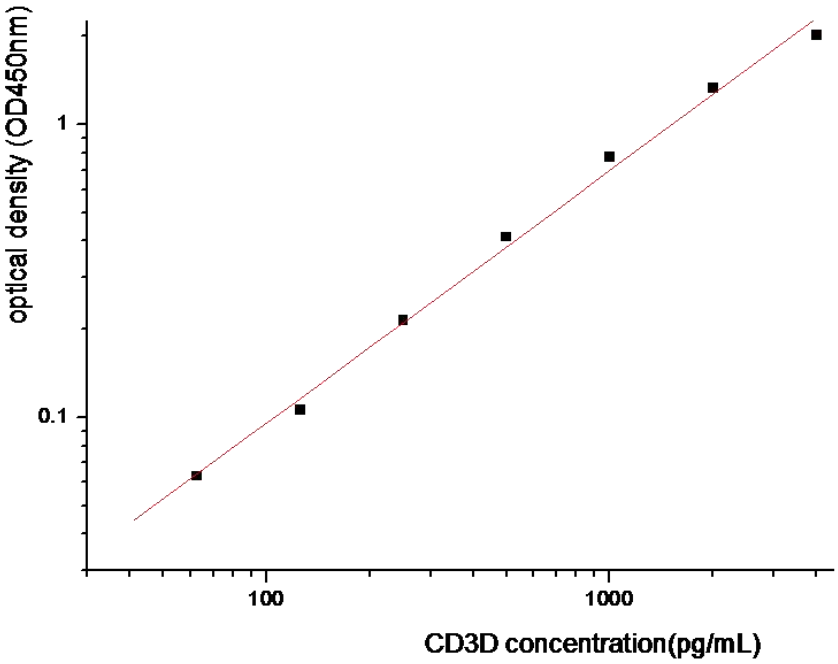
1.Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2.Repeat the aspiration/wash as in step 2 of plate preparation.
3.Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4.Repeat the aspiration/wash as in step 2 of plate preparation.
5.Add 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.
6.Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7.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	0.063
125	0.106
250	0.214
500	0.413
1000	0.771
2000	1.331
4000	2.011

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of human CD3D 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												

Fax :+86-10-58628220 Tel:+86-400-890-9989 http://www.sinobiological.com

Human CD3d / CD3 delta ELISA Pair Set

Notes