Sino Biological
Biological Solution Specialist

# Human CD8A / MAL ELISA Pair Set

## Catalog Number : SEK10980

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

Human T-cell surface glycoprotein CD8 alpha chain, also known as CD8a, is a single-pass type I membrane protein. The CD8 glycoprotein is expressed by thymocytes, mature T cells and natural killer (NK) cells and has been implicated in the recognition of monomorphic determinants on major histocompatibility complex (MHC) Class I antigens, and in signal transduction during the course of T-cell activation. Both human and rodent CD8 antigens are comprised of two distinct polypeptide chains, alpha and beta. The Ig domains of CD8 alpha are involved in controlling the ability of CD8 to be expressed. Mutation of B- and F-strand cysteine residues in CD8 alpha reduced the ability of the protein to fold properly and, therefore, to be expressed. Defects in CD8A are a cause of familial CD8 deficiency. Familial CD8 deficiency is a novel autosomal recessive immunologic defect characterized by absence of CD8+ cells, leading to recurrent bacterial infections.

## PRINCIPLE OF THE TEST

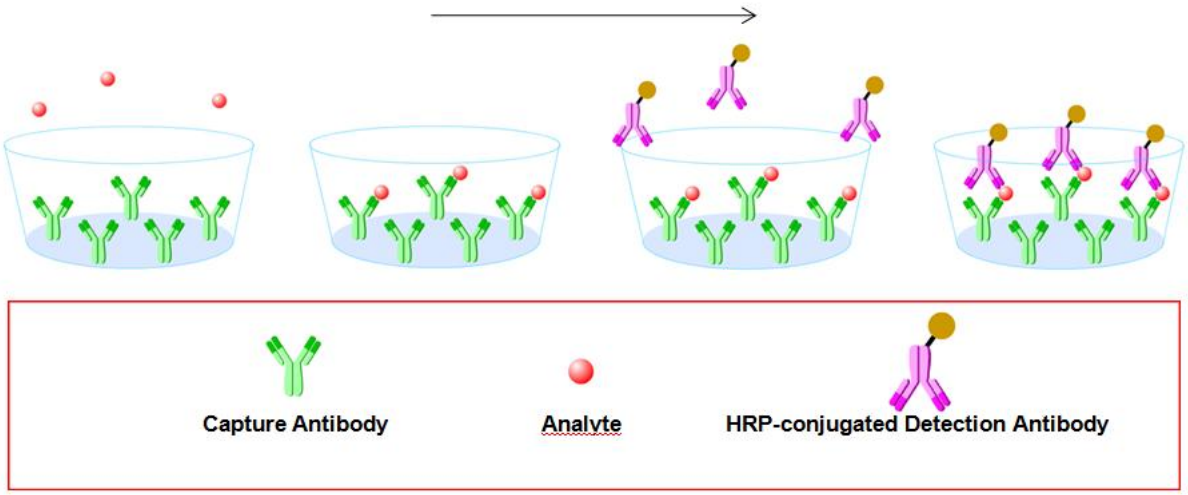
The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for CD8Acoated on a 96-well plate. Standards and samples are added to the wells, andany CD8A present binds to the immobilized antibody. The wells are washed and ahorseradish peroxidase conjugated mouse anti-CD8A monoclonal antibody is thenadded, producing an antibody-antigen-antibody "sandwich". The wells are againwashed and TMB substrate solution is loaded, which produces color in proportionto the amount of CD8A present in the sample. To end the enzyme reaction, thestop solution is added and absorbances of the microwell are read at 450 nm.

## INTENDED USE

◆ The human CD8A ELISA Pair Set is for the quantitative determination of human CD8A .

◆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** – 0.2 mg/mL of mouse anti-CD8A monoclonal antibody. Dilute to a working concentration of 0.5 µg/mL in CBS before coating (Catalog: # 10980-MM01)

**Detection Antibody** – 0.5 mg/mL mouse anti-CD8A monoclonal antibody conjugated to horseradish-peroxidase (HRP) . Dilute to working concentration of 1 µg/mL in detection antibody dilution buffer before use. (Catalog: # 10980-MM07)

**Standard** – Each vial contains 180 ng of recombinant CD8A. 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 5 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

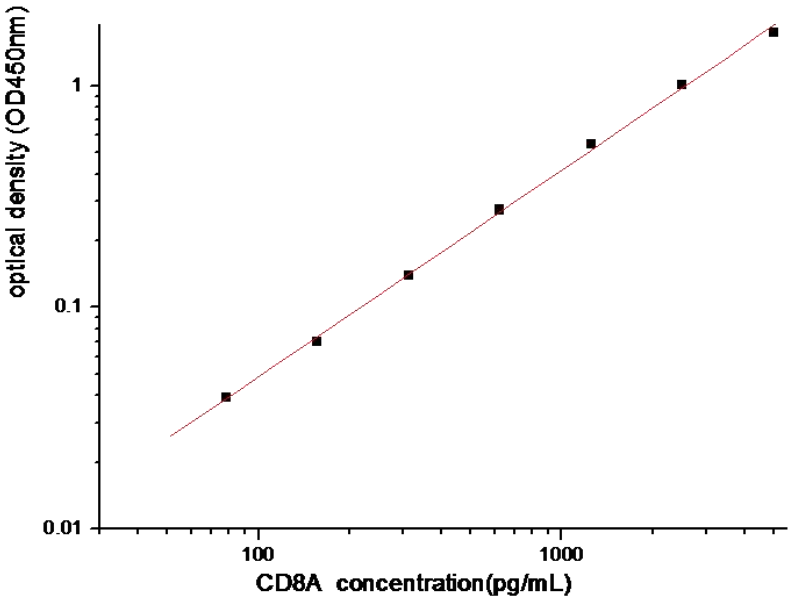
1.Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2.Repeat the aspiration/wash as in step 2 of plate preparation.
3.Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and 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 |
| 78.1 | 0.039 |
| 156.1 | 0.070 |
| 312.5 | 0.140 |
| 625 | 0.276 |
| 1250 | 0.549 |
| 2500 | 1.014 |
| 5000 | 1.742 |

## PERFORMANCE CHARACTERISTIC

### SENSITIVITY

The minimum detectable dose of human CD8A was determined to be approximately 78.1 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 CD8A / MAL ELISA Pair Set

## Notes