

The dual probe Mouse CTLA4/Fc ELISA Kit

Intended Use

The dual probe Mouse CTLA4/Fc ELISA Kit is an enzyme immunoassay for the quantitative determination of mouse CTLA4/Fc in cell culture conditioned medium and serum samples.

The mouse CTLA4/Fc ELISA Kit is for Research Use Only. Not for use in Diagnostic Procedures.

Summary

Principles of the assay:

The dual probe CTLA4/Fc ELISA Kit is a quantitative "sandwich" enzyme immunoassay. A rat IgG antibody specific for mouse CTLA4 has been coated onto the microtiter plates provided in the kit. Test samples or CTLA4/Fc standards are added to the microtiter wells and any CTLA4 present binds to the pre-coated IgG antibody. After washing away unbound sample components, an enzyme-linked rabbit IgG specific for mouse IgG2a Fc is added to the wells. A sandwich is thus formed with the CTLA4/Fc bound by the coated anti-CTLA4 antibody and detecting anti-mouse IgG 2a Fc antibody. Thus the dual probe ELISA ensures this assay specific for mouse CTLA4/Fc fusion protein but neither CTLA4 nor IgG2a. Excess enzyme-conjugated IgG is removed by washing and a substrate solution is added to the wells. Color develops in proportion to the amount of CTLA4/Fc present in the sandwich.

In addition to the samples tested, a Standard Curve is prepared using known concentrations of the CTLA4/Fc standard provided in the kit. The optical density of the test samples is compared with the Standard Curve and the CTLA4/Fc concentration in the sample is determined.

Reagents Provided:

Store all kit components except CTLA4/Fc standard and microtiter plates at 2-8⁰C. Store the CTLA4/Fc standard and microtiter plates at -20⁰C. Spin the vials for a few seconds in a microcentrifuge to ensure that all of the liquid is in the bottom of the tube.

CTLA4/Fc Microtiter Plate- two 96-well microtiter plates coated with a rat IgG antibody specific for mouse CTLA4.

Peroxidase-Conjugated rat IgG antibody - 1 vial (25 µl each) of anti-mouse IgG2a Fc rat IgG conjugated with horseradish peroxidase.

CTLA4/Fc standard - 1 vial (50 µl) of mouse CTLA4/Fc containing 0.5 mg/ml in 1 x PBS.

Please vortex and briefly centrifuge the tube before open it.

30% H₂O₂ – 1 vial (25 µl).

10x Wash Buffer - 1 bottle (100 ml) 10x concentrated solution of buffered surfactant.

Substrate Diluent A - 1 bottles (20 ml) 0.1M citric acid.

Substrate Diluent B - 1 bottles (20 ml) 0.2M Na₂PO₄.

OPD Tablets – 6 tablets of o-phenylenediamine color developer.

0.25M Sulfuric Acid - 1 bottle (15 ml) of 0.25M sulfuric acid Stop Solution. **Caution: caustic material.**

Other Materials are Required:

Spectrophotometer- capable of measurement at 490 nm.

Pipets to deliver 10-100 µl, 1-10 ml solution.

Distilled or deionized water (ddH₂O).
Polypropylene tubes (12x75mm).
Parafilm™.
Wash bottle or other microtiter plate washer.

PRECAUTIONS

Do not expose OPD Tablets or Substrate Diluent to strong light during storage or preparation.
Avoid contact of OPD Tablets, Substrate Diluent and 0.25M Sulfuric Acid with metal objects.
Handle OPD tablets with non-metal forceps or paper toweling.
0.25M Sulfuric Acid is a caustic acid solution. Wear eye, hand, and face protection.

SAMPLES COLLECTION AND STORAGE

Conditioned Culture Medium - Conditioned medium samples should be centrifuged to remove Cellular and particulate material. Store medium samples at -20°C. Avoid multiple freeze-thaw cycles.

REAGENT PREPARATION

BRING ALL REAGENIS TO ROOM TEMPERATURE BEFORE USE. Reagents provided are sufficient to set up two separate assays of up to 192 wells total.

10X Wash Buffer - Dilute 10-fold with distilled or deionized water to prepare 1 liter of wash buffer. Store at 2-8°C.

Microtiter Plate Strips -.Determine the total number of wells needed for the number of samples to be tested in duplicate plus 16 Wells for the Standard Curve.

CTLA4/Fc Standards - The diluent used to dilute CTLA4/Fc Standard to prepare the Standard Curve should be the same culture medium as the sample being tested. Prepare Standards immediately before use in the assay. **Please vortex and briefly centrifuge the tube before open it.** Transfer 1 µl of the stock CTLA4/Fc Standard to a polypropylene tube containing 990 µl of Sample Diluent or other culture medium. Use this 0.5 µg/ml CTLA4/Fc solution to prepare the following CTLA4/Fc Standards:

1. add 100 µl of PBS to each of well of column 1 and 2 of ELISA plate.
2. make 1:3 serial dilution by adding 50 µl of 0.5 µg/ml CTLA4/Fc standard solution to the first wells containing 100 µl PBS and mixing well.
3. then transferring 50 µl of solution from 2nd wells to 3rd wells and mixing well.
4. repeating this dilution until the 8th wells and discard the 50 µl solution.

Peroxidase-(HRPO) Conjugated Anti-IgG2a Fc – Prepare 1:1000 dilution of HRPO anti-IgG2a Fc by adding 11 µl of Ab to 11 ml of PBS.

OPD Substrate Solution: Prepare OPD Substrate Solution 10 minutes before use.

- | | | |
|---|------------------|----------------------------|
| 1. Prepare the substrate solution by mixing | 6.1 ml | Substrate Diluent A |
| | 6.15 ml | Substrate Diluent B |
| | 12.25 ml | ddH₂O |
| | 2 tablets | OPD Tablet |

Keep in dark for 10 minutes.

2. Add 10 µl of 30% H₂O₂ immediately before use..

ASSAY PROCEDURE

Bring all Reagents and Samples to Room Temperature. Mix reagents and samples gently before use.

1. Prepare the duplicate CTLA4/Fc standards as described above and add 100 ul of each Test Sample to duplicate wells of the microtiter plate.
2. Cover the plate with Parafilm™ and incubate for 4 hours at room temperature or overnight at 4⁰C.
3. Aspirate or decant the solution, wash 4 times with wash buffer.
4. Add 100 µl of diluted Peroxidase-Conjugated anti-IgG2a mAb to each well. Cover the plate with Parafilm™ and incubate for 30 minutes at room temperature.
5. Aspirate or decant the solution, wash 5 times with wash buffer.
6. Add 100 µl of OPD substrate Solution to each well, cover -d incubate- in the dark at room temperature for up to 30 minutes.
7. Add 100 µl of Stop Solution (0.25M Sulfuric Acid) to each well.

RESULTS

Plot the absorbance for the Standards versus the concentration of the Standards using linear graph paper draw thin but fit curve.

TYPICAL DATA

Sensitivity - The minimal detectable level of CTLA4/Fc using the Standard Curve generated with Sample Diluent is 100 pg/ml.

Specificity - The mouse CTLA4/Fc ELISA kit is specific for mouse CTLA4/Fc. The following cytokines and immunoglobulin have been tested and shown no/minimum cross-reaction. Mouse IgG2a, IgG1, mouse IL-2, IL-4 and IL-10.

ORDERING INFORMATION

To order please Fax your order to 617 779-0880.

TECHNICAL SERVICES

For technical information., Please call our Technical Services Department at 617-779-8868.

REFERENCES

1. Linsley, P. S., W. Brady, M. Urnes, L. S. Grosmaire, N. K. Damle, and J. A. Ledbetter. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med* 174:561.
2. Lenschow, D. J., T. L. Walunas, and J. A. Bluestone. 1996. CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 14:233.
3. Perez, V. L., L. V. Parijs, A. Biuckans, X. X. Zheng, T. B. Strom, and A. K. Abbas. 1997. Induction of peripheral T cell tolerance in vitro requires CTLA-4 engagement. *Immunity* 6:411.
4. Zheng, X. X., A. W. Steele, P. Nickerson, W. Steurer, J. SteFcer, and T. B. Strom. 1995. Administration of Non-Cytolytic IL-10/Fc in LPS-induced septic shock and allogeneic islet transplantation murine animal models. *J. Immunol.* 154:5590.