ab285331 – Human CTLA-4/CD152 ELISA Kit

For in vitro quantitative determination of CTLA-4 in human serum, plasma, tissue homogenates and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285331

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Anti-human CTLA4 pre-coated Microplate	1	4°C
Lyophilised Standard	2 vials	4°C
Sample and Standard diluent Buffer	20 mL	4°C
Biotin-detection antibody (Concentrated)	120 µL	4°C
Antibody dilution buffer	10 mL	4°C
HRP-Streptavidin Conjugate (SABC)	120 µL	4°C
SABC Dilution buffer	10 mL	4°C
TMB Substrate	10 mL	4°C
Stop Solution	10 mL	4°C
25X Wash Buffer	30 mL	4°C
Plate sealers	5	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.

Biotin- detection antibody working solution: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with 0.1 - 0.2 ml more than the total volume. Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.

HRP-Streptavidin Conjugate (SABC): Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with 0.1 - 0.2 ml more than the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.

Wash Buffer: Dilute wash buffer at 1:25 with distilled water.

Standard Preparation

1) Reconstitute the lyophilized Human CTLA4 standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 10 ng/ml standard stock solution.
2) Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely. Use within 2 hours of reconstituting.
3) Prepare 0.6 ml of 5 ng/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer.
4) Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.

Δ Note: Suggested standard points are: 5, 2.5, 1.25, 0.625, 0.313, 0.156 ng/ml.

Sample Preparation

- Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Cell culture supernatants: Centrifuge to remove precipitate, analyze immediately or aliquot and store at -20°C.

Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 4°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant.

Other biological fluids: Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

Δ Note: *End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.*

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1) Prepare all reagents, samples and standards as instructed.
2) Wash plate 2 times with 1X Wash Solution before adding standard, sample and control wells.

3) Add 100 µl of each standard and samples into appropriate wells. Cover well and incubate for 1.5 hours at 37°C.
4) Remove the cover and discard the plate content without washing or letting the wells completely dry.
5) Add 0.1 ml of Biotin-detection antibody work solution into the above wells. Seal the plate and incubate at 37°C for 60 min.
6) Discard the solution and wash 3 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel Pipette or auto washer. Let it soak for 1 to 2 minutes and then all residual wash-liquid must be drained from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7) Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
8) Discard the solution and wash 5 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel Pipette or auto washer. Let it soak for 1 to 2 minutes and then all residual wash-liquid must be drained from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
9) Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37 °C in dark within 15-30 min.
10) Add 50 µl of Stop Solution to each well. Read result at 450 nm within 20 minutes.

Calculation

- For calculation

(the relative O.D.450) = **(the O.D.450 of each well)** – **(the O.D.450 of Zero well)**.

- The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human CTLA4 concentration of the samples can be interpolated from the standard curve.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information,

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

Copyright © 2021 Abcam, All Rights Reserved. All information / detail is correct at time of going to print.

Version 1, Last updated Tuesday, September 28, 2021