# Human Granzyme B (CTLA-1) ELISA assay

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## Abstract

This protocol describes an ELISA assay for measurement of Granzyme B (CTLA-1) in supernatants collected from T cell dependent cytotoxicity (TDCC) cultures after BiTE® treatment.

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## Protocol

### Reagents

**Step 1.**

- Granzyme B (CTLA-1)Human ELISA Kit, Abcam, P/N ab46142, Lot # GR149011-8
- Granzyme B Microplate (12 x 8 well strips)
- Granzyme B Standard
- 10X Standard Diluent Buffer
- Biotinylated anti-Granzyme B
- Biotinylated Antibody Diluent
- Streptavidin-HRP
- HRP Diluent
- 200X Wash Buffer
- Chromogen TMB Substrate Solution
- Stop Reagent
- Distilled or deionized water

### Prepare solutions

**Step 2.**

1. Prepare Wash Buffer (1X Wash Buffer in DI Water): 1.0 ml 200X Wash buffer + 199 mL Water
2. Prepare 1X Standard Diluent Buffer: Dilute the 10X Standard Diluent Buffer 10-fold in distilled water before use (400 µL 10X Standard Diluent + 3.6 mL distilled water)

### Prepare Standard (calibration curve)

**Step 3.**

Prepare a 1,000 pg/mL **Standard #1** by reconstituting the lyophilized Granzyme B standard with the

volume indicated on the vial using the appropriate 1X Standard Diluent Buffer

| Dilution | Step | | Standard Diluent Buffer (µl) | Conc. (pg/mL) |
|---|---|---|---|---|
| 1:100 | 1 | 500 µl of 1000 pg/mL stock | --- | 1000 |
| 1:2 | 2 | 250 µl of 1000 pg/mL stock | 250 | 500 |
| 1:2 | 3 | 250 µl of 500 pg/mL | 250 | 250 |
| 1:2 | 4 | 250 µl of 250 pg/mL | 250 | 125 |
| 1:2 | 5 | 250 µl of 125 pg/mL | 250 | 62.5 |
| 1:2 | 6 | 250 µl of 62.5 pg/mL | 250 | 31.3 |
| 1:2 | 7 | 250 µl of 31.3 pg/mL | 250 | 15.6 |
| 1:2 | 8 | --- | 250 | 0 |

## Prepare 1X Biotinylated anti-Granzyme B

**Step 4.**

| Number of well strips used | Volume of Biotinylated anti-Granzyme B (µL) | Volume of Biotinylated Antibody Diluent (µL) |
|---|---|---|
| **4** | **80** | **2,120** |

## Add standards to plate

**Step 5.**

Add 100 µl of Standard to the appropriate wells of the plate

## Add samples to plate

**Step 6.**

Add 100 µl of Neat Sample to the appropriate wells of the plate

## Add biotinylated antibody solution

**Step 7.**

1. Add 50 µl of Biotin Antibody Solution to each well
2. Seal the plate
3. Cover with a plate sealer
4. Incubate for 3 hours at room temperature

## Prepare 1X Streptavidin-HRP Solution

**Step 8.**

Add 500 µl to the vial

| Number of well strips used | Volume of Streptavidin-HRP (µL) | Volume of HRP-Diluent (mL) |
|---|---|---|
| **4** | **60** | **4** |

## Wash plate

**Step 9.**

1. After incubation, wash the plate 3X with 1X Wash Buffer using a plate washer

2. Aspirate the liquid from each well.
3. Add 0.3 µL of 1X Wash Buffer into each well
4. Aspirate the liquid from each well.
5. Repeat for a total of 3 washes.
6. Blot plate on a paper towel

## Add Streptavidin Solution to plate

**Step 10.**

1. Add 100 µl of Streptavidin Solution to each well
2. Seal the plate
3. Cover with aluminum foil
4. Incubate for 30 minutes at room temperature

## Wash plate

**Step 11.**

1. After incubation, wash the plate 3X with Wash Buffer using a plate washer
2. Aspirate the liquid from each well.
3. Add 0.3 µL of 1X Wash Buffer into each well
4. Aspirate the liquid from each well.
5. Repeat for a total of 3 washes.
6. Blot plate on a paper towel

## Add substrate to plate

**Step 12.**

1. Add 100 µl Chromogen TMB substrate solution to each well of the plate
2. Incubate in the dark for 10-20 minutes at room temperature.
3. Avoid direct exposure to light by wrapping the plate in aluminum foil.

## Add Stop Solution to plate

**Step 13.**

Add 100 µl Stop Solution to each well of the plate

## Read plate

**Step 14.**

1. Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength
2. Optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

## Data analysis

**Step 15.**

Calculate the mean absorbance for each set of duplicate standards and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.