Human FASL ELISA assay

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Abstract

This protocol describes an ELISA assay for measurement of FAS ligand (FASL, CD95L) in supernatants collected from T cell dependent cytotoxicity (TDCC) cultures after BiTE® treatment.

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Protocol

Reagents

Step 1.

- FAS Ligand (CD95L)Human ELISA Kit, Abcam, P/N ab45892, Lot # GR141964-4
- FAS Ligand (CD95L) Microplate (12 x 8 well strips)
- FAS Ligand (CD95L)Standard
- 10X Standard Diluent Buffer
- Biotinylated anti-FAS Ligand (CD95L)
- Biotinylated Antibody Diluent
- Streptavidin-HRP
- HRP Diluent
- 200X Wash Buffer
- Chromogen TMB Substrate Solution
- Stop Reagent
- Distilled or deionized water

Prepare reagents

Step 2.

1. Prepare Wash Buffer (1X Wash Buffer in Di Water): 1.0 ml 200X Wash buffer + 199 mL DI water
2. Prepare 1X Standard Diluent Buffer: Dilute the 10X Standard Diluent Buffer 10-fold in distilled water before use (0.4 mL 10X Standard Diluent + 3.6 mL distilled water)

Prepare standards (calibration curve)

Step 3.

1. Prepare a 1,000 pg/mL **Standard #1** by reconstituting the lyophilized FASL standard with the volume indicated on the vial using the appropriate 1X Standard Diluent Buffer

Dilution	Step		Standard Diluent Buffer (uL)	Conc. (pg/mL)
1:100	1	500 uL of 2000 pg/mL stock	---	2000
1:2	2	250 uL of 2000 pg/mL	250	1000
1:2	3	250 uL of 1000 pg/mL	250	500
1:2	4	250 uL of 500 pg/mL	250	250
1:2	5	250 uL of 250 pg/mL	250	125
1:2	6	250 uL of 125 pg/mL	250	62.5
1:2	7	250 uL of 62.5 pg/mL	250	31.3
1:2	8	---	250	0

Add standards to plate

Step 4.

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

Add samples to plate

Step 5.

1. Add 100 µl of neat sample to the appropriate wells of the plate
2. Seal the plate
3. Incubate for 2 hours at room temperature

Prepare 1X biotinylated anti-FASL

Step 6.

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

Wash plate

Step 7.

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 biotinylated antibody

Step 8.

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

Prepare Streptavidin-HRP Solution

Step 9.

1. Add 500 µL to the vial
2. Prepare 1X Streptavidin-HRP Solution

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

Wash plate

Step 10.

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

Step 11.

1. Add 100 uL of Streptavidin Solution to each well
2. Seal the plate
3. Cover with aluminum foil
4. Incubate for 20 minutes at room temperature

Wash plate

Step 12.

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

Step 13.

1. Add 100 µL Chromogen TMB substrate solution to each well of the plate
2. Incubate in the dark for 15-20 minutes at room temperature.
3. Avoid direct exposure to light by wrapping the plate in aluminum foil.

Add Stop Solution

Step 14.

Add 100 µL Stop Solution to each well of the plate

Read plate

Step 15.

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 16.

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.