MSD 96-well human cytokine assay

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Abstract

This protocol describes MesoScale Discovery (MSD) assays for measurement of proinflammatory cytokines in supernatants collected from T cell dependent cytotoxicity (TDCC) cultures after BiTE® treatment.

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Protocol

Reagents

Step 1.

- Human Proinflammatory-4 I Tissue Culture Kit, MSD, P/N K15009B-2, Lot # K0034148
- Human Proinflammatory-4 I Plate, MSD, P/N N45009B-1, Lot # Z0044786
- Diluent 1, MSD, P/N R50CK-4, Lot #Y0220021
- Diluent 100, MSD, P/N R50AA-4, Lot #Y0270189
- MSD Blocker B, P/N R93BB-2, Lot # Y0210033
- Human Proinflammatory-4 I Antibody Blend, MSD, P/N D2009-3, Lot # D0032825
- Human Proinflammatory-4 I Calibrator Blend, MSD, P/N C0009-2, Lot # A0031804
- PBST (PBS + 0.05% Tween 20)
- DPBS, Life, P/N 14190

Prepare solutions

Step 2.

1. Wash Buffer (PBS + 0.05% Tween 20): 2L (2000mL) PBS + 1.0 mL Tween20
2. Prepare Blocker B Solution (1% w/v Blocker B in PBS): 20 mL PBS + 200 mg Blocker B

Block plates

Step 3.

1. Add 150 uL of Blocker B Solution (Step 2) to each well of the pre-coated plate
2. Seal the plate
3. Incubate for 1 hour at room temperature, with shaking at 800 rpm

Prepare standard (calibration curve)

Step 4.

Dilution	Step	Volume Diluent 1 (uL)	Conc. (pg/mL)

1:100	1	10 uL of 1.0 ug/mL stock	990	10000
1:4	2	50 uL of 10 ng/mL (1)	150	2500
1:4	3	50 uL of 2.5 ng/mL (2)	150	625
1:4	4	50 uL of 625 ng/mL (3)	150	156
1:4	5	50 uL of 156 pg/mL (4)	150	39
1:4	6	50 uL of 39 pg/mL (5)	150	9.8
1:4	7	50 uL of 9.8 pg/mL (6)	150	2.4
1:4	8	---	200	0

Wash blocked plate

Step 5.

1. Wash the plate 3X with PBST using a plate washer

2. Blot plate on a paper towel

Add sample and standards to plate

Step 6.

1. Add 25 µL of neat sample and standards to the appropriate wells of the plate
2. Seal the plate
3. Incubate for 2 hours at room temperature, with shaking at 800 rpm

Prepare Detection Antibody solution (1X in PBS)

Step 7.

1. 2.94 mL Diluent 100 + 60 µL 50X Detection Antibody
2. Keep in the dark

Add Detection ntibody solution to each well

Step 8.

1. 25 µL of Detection Antibody Solution to each well
2. Seal the plate
3. Cover with aluminum foil
4. Incubate for 2 hours at room temperature, with shaking at 800 rpm

Prepare 2X Read Buffer Solution

Step 9.

11 mL DI H20 + 11 mL 4X Read Buffer

Wash plates

Step 10.

1. After incubation, wash the plate 3X with PBST using a plate washer
2. Blot plate on a paper towel

Add Read Buffer Solution to each well

Step 11.

1. Add 150 µL 2X Read Buffer solution to each well of the MSD plate
2. Make sure there are NO BUBBLES
3. Read immediately on MSD instrument

Analyze data

Step 12.

Analyze data using MSD software