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MSD V-PLEX Proinflammatory panel

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We use this protocol and it's working

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Abstract

The V-PLEX panel offers analytically validated multiplex assay kits. Developed under rigorous design control, V-PLEX kits provide accurate and reproducible results with consistency from lot to lot. The V-PLEX Proinflammatory Panel 1 (human) Kit includes ten biomarkers that are associated with the inflammatory response and immune system regulation.

MSD V-PLEX Proinflammatory Panel

1 Prepare calibrator dilutions

a. Prepare the highest calibrator (Calibrator 1) by adding 1000mL of Diluent 2 to the lyophilized calibrator vial. After reconstituting, invert at least 3 times. Let it equilibrate for 15-30 minutes at room temperature and then vortex briefly using short pulses.

b. Prepare the next calibrator by transferring 100mL to the highest calibrator to 300mL of Diluent 2.

c. Use Diluent 2 as the zero calibrator.

2 Prepare Detection Antibody Solution

For one plate, combine 2.400mL of Diluent 3 with:

- 60mL of SULFO-TAG Anti-hu IFN-γ Antibody
- 60mL of SULFO-TAG Anti-hu IL-1b Antibody
- 60mL of SULFO-TAG Anti-hu IL-2 Antibody
- 60mL of SULFO-TAG Anti-hu IL-4 Antibody
- 60mL of SULFO-TAG Anti-hu IL-6 Antibody
- 60mL of SULFO-TAG Anti-hu IL-8 Antibody
- 60mL of SULFO-TAG Anti-hu IL-10 Antibody
- 60mL of SULFO-TAG Anti-hu IL-12p70 Antibody
- 60mL of SULFO-TAG Anti-hu IL-13 Antibody
- 60mL of SULFO-TAG Anti-hu TNF-α Antibody

3 Prepare Wash Buffer

For one plate combine:

- 15mL of MSD Wash Buffer (20X)
- 285mL of deionized water

4 Prepare Read Buffer T

For one plate combine:

- 10mL of Read Buffer T (4X)
- 10mL of deionized water

5 Assay Protocol

5.1 Wash and Add Sample

a. Wash the plate 3 times with at least 150mL/well of Wash Buffer.

b. Add 50uL of prepared samples, calibrators, or controls per well.

c. Seal the plate with an adhesive plate seal.

d. Incubate at room temperature with shaking for 2h.

5.2 Wash and Add Detection Antibody Solution

a. Wash the plate 3 times with at least 150mL/well of Wash Buffer.

b. Add 25uL of detection antibody solution to each well.
c. Seal the plate with an adhesive plate seal.
d. Incubate at room temperature with shaking for 2h.

5.3 Wash and Read

a. Wash the plate 3 times with at least 150mL/well of Wash Buffer.
b. Add 150uL of 2X Read Buffer T to each well.
c. Seal the plate with an adhesive plate seal.
d. Analyze the plate on an MSD instrument.