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#### 1. Description

This product is for research use only.

**Components** 1 vial MACSplex Cytokine 12 Standard, human: Lyophilized mixture of recombinant GM-CSF, IFN- $\alpha$ , IFN- $\gamma$ , IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-17A, and TNF- $\alpha$ .

**Size** For 2 standard curves.

**Product format** MACSplex Cytokine 12 Standard contains stabilizer.

**Storage** Store protected from light at  $-20^{\circ}\text{C}$ . The expiration date is indicated on the vial label.

##### 1.1 Background information

MACSplex Assays have been designed for determining concentrations of soluble analytes in a single sample. The analysis is based on MACSplex Capture Beads, which display defined fluorescence properties and can be identified using standard flow cytometry techniques.

The MACSplex Cytokine 12 Standard in combination with MACSplex Cytokine Reagents Kits, human and the MACSplex Cytokine Basic Kit allows the simultaneous flow cytometric detection of up to seven soluble human cytokines in a single sample.

##### 1.2 Applications

- The MACSplex Cytokine 12 Standard has been developed as a standard control for the quantification of the analytes within the unknown samples.

##### 1.3 Reagent and instrument requirements

- MACSplex Cytokine Basic Kit (# 130-109-701)
- Up to seven MACSplex Cytokine Reagent Kits, human of choice.
- MACSQuant<sup>®</sup> Analyzer, MACSQuant Analyzer 10 (# 130-096-343), or other flow cytometers equipped with blue (488 nm) and red (635 nm) lasers able to discriminate FITC, PE, and APC fluorescence.

▲ **Note:** The MACSQuant VYB cannot be used.

#### 2. Protocol

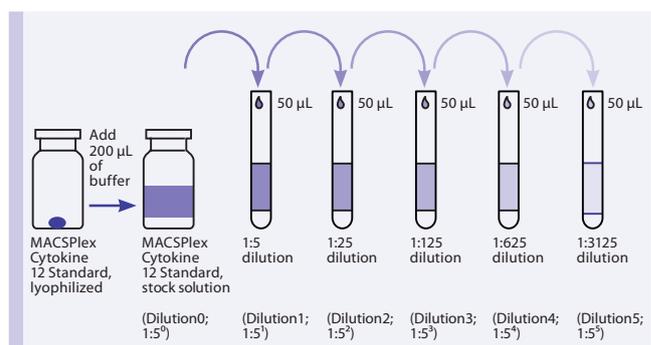
▲ Reconstitute and dilute MACSplex Cytokine 12 Standard with MACSplex Buffer provided in the MACSplex Cytokine Basic Kit, or use the same media as is used for the dilution of the unknown sample.

▲ Only use freshly prepared MACSplex Cytokine 12 Standard solutions. Do not store or reuse reconstituted or diluted standards.

▲ Use polypropylene or polystyrene reagent tubes. Do not use glass vials.

The generation of standard curves requires seven samples: six samples ranging from 3.2 to 10,000 pg/mL of the MACSplex Cytokine 12 Standard, and one blank control. These samples will be measured as duplicates.

1. Thaw one vial containing the lyophilized MACSplex Cytokine 12 Standard.
2. Open the vial and add 200  $\mu\text{L}$  of MACSplex Buffer or media to the pellet. Mix gently. This is the stock solution (1:5<sup>0</sup>; 10,000 pg/mL).
3. Label five reagent tubes and arrange them in the following order: 1:5 (1:5<sup>1</sup>; 2,000 pg/mL), 1:25 (1:5<sup>2</sup>; 400 pg/mL), 1:125 (1:5<sup>3</sup>; 80 pg/mL), 1:625 (1:5<sup>4</sup>; 16 pg/mL), and 1:3125 (1:5<sup>5</sup>; 3.2 pg/mL).
4. Pipette 200  $\mu\text{L}$  of MACSplex Buffer or media into each tube.
5. Perform a 1:5 dilution by transferring 50  $\mu\text{L}$  from the stock solution to the tube labeled 1:5 and mix thoroughly. Continue making 1:5 serial dilutions by transferring 50  $\mu\text{L}$  from the tube labeled 1:5 to the tube labeled 1:25 and so on to the tube labeled 1:3125. Mix each dilution before performing the next transfer.
6. Keep 200  $\mu\text{L}$  MACSplex Buffer or media as blank control (0 pg/mL).



**Figure 1:** Serial dilution of the MACSplex Cytokine 12 Standard.

7. Perform the MACSplex Cytokine Assay according to the protocol of the MACSplex Cytokine Basic Kit.

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