

## Contents

### 1. Description

- 1.1 Background information
- 1.2 Application
- 1.3 Reagent and instrument requirements

### 2. Protocol

## 1. Description

**This product is for research use only.**

Components	1 vial MACSPlex Mix Cytotoxic Standard, human: Lyophilized mixture of recombinant GM-CSF, Granzyme B, IFN- $\gamma$ , IL-2, IL-4, IL-6, IL-10, IL-17A, IL-21, MCP-1, Perforin, and TNF- $\alpha$ .
Size	For 2 standard curves.
Product format	MACSPlex Mix Cytotoxic Standard, human 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 Mix Cytotoxic Standard, human in combination with MACSPlex Mix Cytotoxic Reagents, human, and the MACSPlex Mix Cytotoxic Basic Kit allows the simultaneous flow cytometric detection of up to twelve soluble human cytokines in a single sample.

### 1.2 Application

- The MACSPlex Mix Cytotoxic Standard, human has been developed as a standard control for the quantification of analytes within unknown samples.

### 1.3 Reagent and instrument requirements

- MACSPlex Mix Cytotoxic Basic Kit (# 130-125-767).
- Up to twelve MACSPlex Mix Cytotoxic Reagents, human of choice.
- MACSQuant® 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.

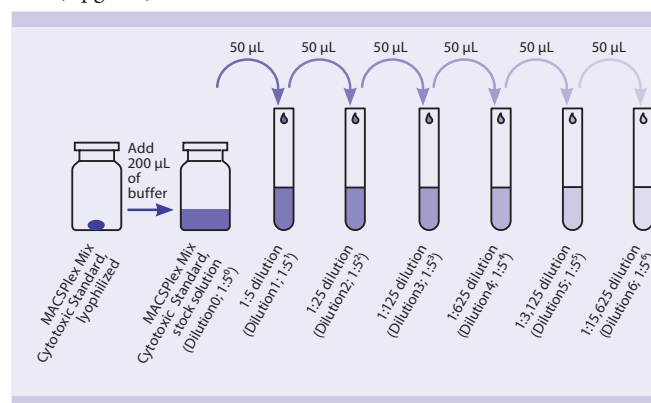
▲ **Note:** The MACSQuant VYB cannot be used.

## 2. Protocol

- ▲ Reconstitute and dilute MACSPlex Mix Cytotoxic Standard, human with MACSPlex Buffer, or use the same media as is used for the dilution of the unknown sample.
- ▲ Only use freshly prepared MACSPlex Mix Cytotoxic Standard, human 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 eight samples: seven samples of the MACSPlex Mix Cytotoxic Standard, human and one blank control.

1. Thaw one vial containing the lyophilized MACSPlex Mix Cytotoxic Standard, human.
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 (10,000 pg/mL for IL-6, MCP-1 (CCL2), IL-2, GM-CSF, and IL-17A and 50,000 pg/mL for Granzyme B, Perforin, IFN- $\gamma$ , IL-21, IL-10, IL-4, and TNF- $\alpha$ ).
3. Label six reagent tubes and arrange them in the following order: 1:5 (1:5<sup>1</sup>; 2,000 pg/mL or 10,000 pg/mL), 1:25 (1:5<sup>2</sup>; 400 pg/mL or 2,000 pg/mL), 1:125 (1:5<sup>3</sup>; 80 pg/mL or 400 pg/mL), 1:625 (1:5<sup>4</sup>; 16 pg/mL or 80 pg/mL), 1:3,125 (1:5<sup>5</sup>; 3.2 pg/mL or 16 pg/mL), and 1:15,625 (1:5<sup>6</sup>; 0.6 pg/mL or 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 1:15,625. 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 Mix Cytotoxic Standard.

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