Miltenyi Biotec

MACSPlex Mix Cytotoxic Reagents
human

Contents

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components MACSPlex Mix Cytotoxic Reagents, human contains 1 vial of MACSPlex Mix Cytotoxic Capture Beads, human and 1 vial of MACSPlex Cytotoxic Detection Reagent 1, human for separate detection of following analytes:

Cytokine	Order no.
GM-CSF	130-125-819
Granzyme B	130-125-818
IFN-γ	130-125-813
IL-2	130-125-968
IL-4	130-125-836
IL-6	130-125-837
IL-10	130-125-838
IL-17A	130-125-841
IL-21	130-125-815
MCP-1	130-125-816
Perforin	130-125-814
TNF-α	130-125-806

Size up to 100 tests

Product format All products are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration dates are indicated on the vial labels.

1.1 Principle of MACSPlex Mix Cytotoxic Reagents, human

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

MACSPlex Mix Cytotoxic Reagents, human contains the MACSPlex Mix Cytotoxic Capture Beads and the MACSPlex Cytotoxic Detection Reagent 1 for the detection of one of the following cytokine specificity: GM-CSF, Granzyme B, IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17A, IL-21, MCP-1, Perforin, and TNF-α. MACSPlex Mix Cytotoxic Capture Beads within a kit contain a single fluorescently labeled bead population, coated with a specific antibody reacting with one of the analytes within the sample. Each bead population can be distinguished by different fluorescence intensities detected in the B1 and B2 channel of the MACSQuant® Analyzer and MACSQuant Analyzer 10.

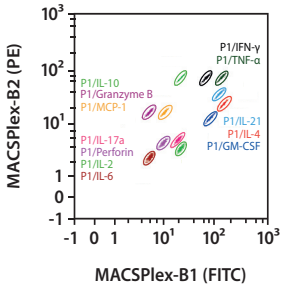

Figure 1: Detection of MACSPlex Mix Cytotoxic Capture Bead populations in a MACSPlex-B1 (FITC) versus MACSPlex-B2 (PE) dot plot.

Two detection reagents, one composed of a cocktail of Biotin-conjugated antibodies specific for the analytes and one composed of Biotin-APC antibodies, are added. Consequently, sandwich complexes are formed between the MACSPlex Mix Cytotoxic Capture Bead, the analyte, and the detection reagent.
For a protocol of the MACSPlex Cytotoxic Assay refer to the data sheet of the MACSPlex Mix Cytotoxic Basic Kit.

1.3 Application

- MACSPlex Mix Cytotoxic Reagents, human has been designed for detection of up to twelve analytes of choice in a single sample of the soluble human cytokines GM-CSF, Granzyme B, IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17A, IL-21, MCP-1, Perforin, and TNF-α. The reagents have been optimized for use with serum, plasma, and cell culture supernatants.

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140-005-695.01

1.4 Reagent requirements

- MACSPlex Mix Cytotoxic Standard, human (# 130-125-766)
- MACSPlex Mix Cytotoxic Basic Kit (# 130-125-767)
- 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. Performance

2.1 Theoretical limit of detection

The assay sensitivity, or theoretical limit of detection, was determined as the concentration corresponding to a median fluorescence intensity (MFI), which is two standard deviations above the mean of MFIs of 26 replicates of negative controls (0 pg/mL). Standard curves were calculated from duplicate standards with a five parameter logistic curve fit.

Cytokine	Limit of detection [pg/mL]
GM-CSF	0.10
Granzyme B	2.47
IFN-γ	0.62
IL-2	0.35
IL-4	21.91
IL-6	0.05
IL-10	1.73
IL-17A	0.12
IL-21	16.06
MCP-1 (CCL2)	0.59
Perforin	93.75
TNF-α	0.12

2.2 Intra-assay precision

To confirm the reproducibility of the MACSPlex Mix CytotoxicAssay, within one assay four replicates of three different concentrations of each cytokine were tested. The assay was carried out including two standard curves. The table below shows the mean, the standard deviation, and the coefficient of variation for each sample.

Cytokine	Sample	Mean (pg/mL)	Standard Deviation	%CV
GM-CSF	Sample 1	79.4	3.4	4.3
	Sample 2	355.3	2.5	0.7
	Sample 3	1951.2	36.2	1.9
Granzyme B	Sample 1	80.3	6.0	7.4
	Sample 2	426.4	5.9	1.4
	Sample 3	2087.9	39.8	1.9
IFN-γ	Sample 1	89.1	16.9	19.0
	Sample 2	512.1	57.7	11.3
	Sample 3	2176.2	138.5	6.4
IL-2	Sample 1	74.3	1.5	2.0
	Sample 2	355.2	7.2	2.0
	Sample 3	1895.2	54.5	2.9
IL-4	Sample 1	79.1	9.6	12.1
	Sample 2	511.6	69.9	13.7
	Sample 3	2230.7	96.9	4.3
IL-6	Sample 1	77.6	1.1	1.4
	Sample 2	374.1	7.2	1.9
	Sample 3	1986.8	93.5	4.7
IL-10	Sample 1	75.3	2.5	3.4
	Sample 2	378.7	11.3	3.0
	Sample 3	1895.2	10.0	0.5
IL-17A	Sample 1	76.1	2.2	2.9
	Sample 2	363.3	3.8	1.1
	Sample 3	2077.5	278.4	13.4
IL-21	Sample 1	67.5	15.5	22.9
	Sample 2	466.8	38.8	8.3
	Sample 3	2206.3	66.8	3.0
MCP-1 (CCL2)	Sample 1	76.4	1.1	1.4
	Sample 2	365.0	4.2	1.1
	Sample 3	2085.0	123.4	5.9
Perforin	Sample 1	*	–	–
	Sample 2	349.3	23.1	6.6
	Sample 3	1659.6	55.8	3.4
TNF-α	Sample 1	82.5	5.5	6.7
	Sample 2	390.9	8.3	2.1
	Sample 3	1850.1	15.8	0.9

2.3 Inter-assay precision

To assess the assay-to-assay reproducibility, two different concentrations of each cytokine were tested in five independent experiments. Each assay was carried out including two standard curves on each plate and two replicates of samples. The table below shows the mean, the standard deviation, and the coefficient of variation for each sample.

Cytokine	Sample	Mean (pg/mL)	Standard Deviation	%CV
GM-CSF	Sample 1	81.2	7.0	8.7
	Sample 2	394.2	23.8	6.0
Granzyme B	Sample 1	425.6	64.2	15.1
	Sample 2	2158.2	217.9	10.1
IFN-γ	Sample 1	442.7	67.7	15.3
	Sample 2	2424.5	277.9	11.5
IL-2	Sample 1	79.8	7.6	9.5
	Sample 2	394.7	24.9	6.3
IL-4	Sample 1	426.2	132.3	31.0
	Sample 2	2455.3	375.1	15.3
IL-6	Sample 1	79.9	7.8	9.7
	Sample 2	404.9	21.2	5.2
IL-10	Sample 1	398.5	41.3	10.4
	Sample 2	2048.4	112.1	5.5
IL-17A	Sample 1	78.9	8.2	10.5
	Sample 2	398.2	24.4	6.1
IL-21	Sample 1	454.9	83.2	18.3
	Sample 2	2417.4	305.7	12.6
MCP-1 (CCL2)	Sample 1	79.6	6.6	8.2
	Sample 2	404.0	26.3	6.5
Perforin	Sample 1	414.6	80.4	19.4
	Sample 2	2016.8	125.4	6.2
TNF-α	Sample 1	401.3	43.8	10.9
	Sample 2	1995.6	136.5	6.8

Specificity

Cross-reactivity of the antibodies against the analytes was tested. No cross-reactivity was observed.

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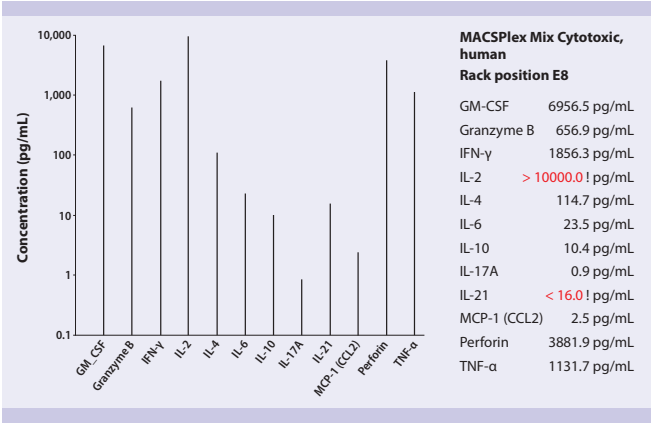
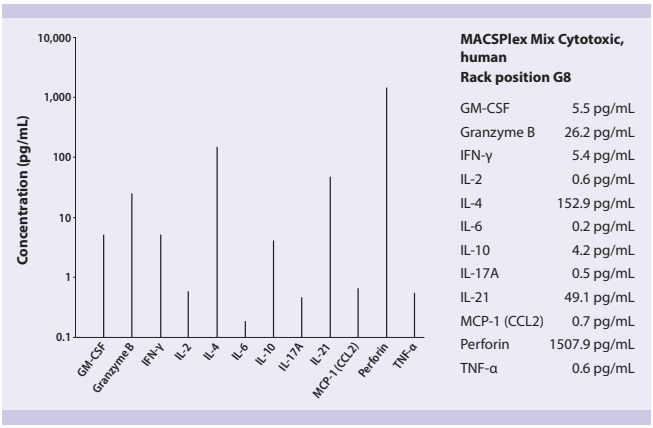
2.4 Exemplary results of the analysis using the Express Mode

A: Co-culture of transduced CD19 CAR T cells and tumor cells (Jeko-1)



B: Culture of transduced CD19 CAR T cells



C: Co-culture of non-transduced T cells and tumor cells (Jeko-1)

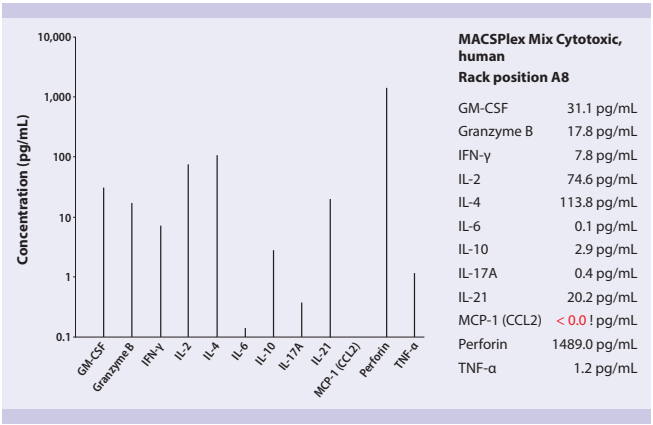


Figure 2: Transduced CD19 CAR T cells were co-cultivated overnight with tumor cells (Jeko-1) in TexMACS™ Medium (A). As a control, transduced CD19 CAR T cells were cultivated without tumor cells (Jeko-1) (B) and non-transduced T cells were co-cultivated with tumor cells (Jeko-1) (C). Cell culture supernatants were harvested and centrifuged to remove particulates. Undiluted samples were analyzed using the MACSPlex Cytotoxic T/NK Cell Kit, human. Two standard curves were carried out and the concentrations of all 12 analytes were determined using the MACSQuant® Express Mode.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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