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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 Each MACSplex Cytokine Reagents Kit, human contains 1 vial of MACSplex Cytokine Capture Beads, human and 1 vial of MACSplex Cytokine Detection Reagent, human for separate detection of following analytes:

Cytokine	Order no.
GM-CSF	130-109-684
IFN- α	130-109-683
IFN- γ	130-109-695
IL-2	130-109-704
IL-4	130-109-689
IL-5	130-109-705
IL-6	130-109-691
IL-9	130-109-693
IL-10	130-109-699
IL-12p70	130-109-697
IL-17A	130-109-688
TNF- α	130-109-694

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 Cytokine Reagents Kits, human

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

Each MACSplex Cytokine Reagents Kit contains the MACSplex Cytokine Capture Beads and the MACSplex Cytokine Detection Reagent for the detection of one of the following cytokine specificity: GM-CSF, IFN- α , IFN- γ , IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-17A, and TNF- α . MACSplex Cytokine Capture Beads within a kit contain a single fluorescently labeled bead populations, 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.

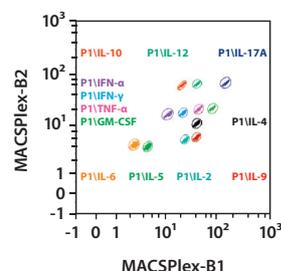


Figure 1: Detection of 12 MACSplex Cytokine Capture Bead populations specific for human cytokine in a MACSplex-B1 (FITC) versus MACSplex-B2 (PE) dot plot.

MACSplex Cytokine Capture Beads are added to both, the unknown samples and to the serial dilutions of the MACSplex Cytokine Standard. During a 2-hour incubation period, the cytokines are captured by the MACSplex Capture Beads. Subsequently, the MACSplex Cytokine Detection Reagent containing an APC-conjugated antibody specific for one analyte, is added in order to form sandwich complexes during a 1-hour incubation period. Standard curves for each cytokine are generated. The median of the APC fluorescence of each capture bead population gives the concentration of each cytokine in the unknown samples.

For a protocol of the MACSplex Cytokine Assay refer to the data sheet of the MACSplex Cytokine Basic Kit.

1.2 Applications

- MACSplex Cytokine Reagents Kits have been designed for detection of up to seven analytes of choice in a single sample of the soluble human cytokines GM-CSF, IFN- α , IFN- γ , IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-17A, and TNF- α . The kits have been optimized for use with serum, plasma, and cell culture supernatants.

1.3 Reagent requirements

- MACSPlex Cytokine 12 Standard, human (# 130-106-197)
- MACSPlex Cytokine Basic Kit (# 130-109-701)
- 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 23 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	0.20
IFN- α	10.4
IFN- γ	6.26
IL-2	1.52
IL-4	34.2
IL-5	0.27
IL-6	0.077
IL-9	32.7
IL-10	2.76
IL-12	3.44
IL-17A	0.7
TNF- α	2.17

2.2 Intra-assay precision

To confirm the reproducibility of the MACSPlex Cytokine Assay 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	74.6	1.8	2.4
	Sample 2	369.8	20.7	5.6
	Sample 3	1841.7	42.1	2.3
IFN- α	Sample 1	76.4	6.4	8.3
	Sample 2	373.3	18.2	4.9
	Sample 3	1742.3	80.8	4.6
IFN- γ	Sample 1	67.3	3.6	5.4
	Sample 2	351	41.5	11.8
	Sample 3	1805.2	51.8	2.9
IL-2	Sample 1	75.1	3.8	5.1
	Sample 2	379.5	19.8	5.2
	Sample 3	1783.3	54.7	3.1
IL-4	Sample 1	368.8	44.9	12.2
	Sample 2	1867.7	96.3	5.2
	Sample 3	9343.4	410.3	4.4
IL-5	Sample 1	60	5.2	8.6
	Sample 2	358	14.1	3.9
	Sample 3	1806.6	93.6	5.2
IL-6	Sample 1	74.1	0.9	1.2
	Sample 2	372.3	11.6	3.1
	Sample 3	1829.5	43.7	2.4
IL-9	Sample 1	84.9	4.5	5.3
	Sample 2	365.4	21.5	5.9
	Sample 3	1837.1	84.2	4.6
IL-10	Sample 1	66.4	3.9	5.9
	Sample 2	345.6	23.4	6.8
	Sample 3	1802	38.5	2.1
IL-12	Sample 1	70.7	5.2	7.3
	Sample 2	380.1	32.7	8.6
	Sample 3	1909.2	42.5	2.2
IL-17A	Sample 1	72.3	1.9	2.7
	Sample 2	389.8	17.2	4.4
	Sample 3	1756.4	161.4	9.2
TNF- α	Sample 1	72.6	2.3	3.2
	Sample 2	353.2	35.8	10.1
	Sample 3	1801.5	67.9	3.8

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	413.5	14.8	3.6
	Sample 2	2080.9	122.4	5.9
IFN- α	Sample 1	206.4	20.1	9.7
	Sample 2	1324.1	123.4	9.3
IFN- γ	Sample 1	110.2	10.6	9.6
	Sample 2	591.4	79	13.4
IL-2	Sample 1	362.4	10.8	3
	Sample 2	1835.3	43	2.3
IL-4	Sample 1	433.3	71.3	16.5
	Sample 2	2259.6	239.7	10.6
IL-5	Sample 1	104.3	17.1	16.4
	Sample 2	590.6	38	6.4
IL-6	Sample 1	373.2	16.3	4.4
	Sample 2	1817.1	37.9	2.1
IL-9	Sample 1	503.2	85.1	16.9
	Sample 2	3040.4	373.6	12.3
IL-10	Sample 1	130.7	6.5	5
	Sample 2	703.1	22.8	3.2
IL-12	Sample 1	261.9	13.9	5.3
	Sample 2	1271.1	40.9	3.2
IL-17A	Sample 1	219.8	11.8	5.4
	Sample 2	1414.3	125.2	8.9
TNF- α	Sample 1	268.5	11.8	4.4
	Sample 2	1362.9	45	3.3

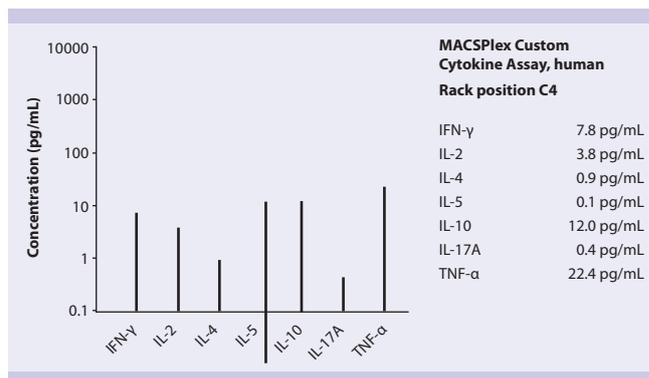
Specificity

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

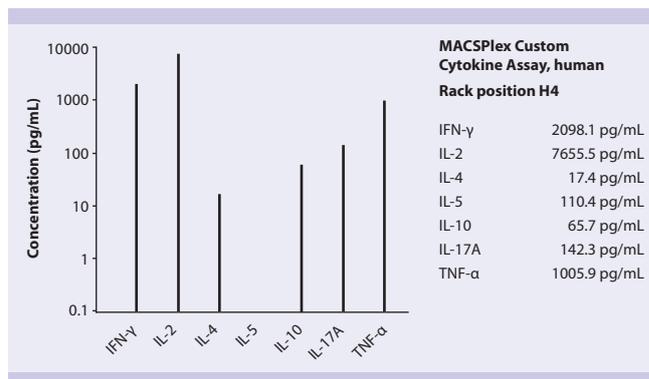
2.4 Exemplary results of the analysis using the Express Mode

CD4⁺ T cells, isolated from peripheral blood mononuclear cells (PBMCs) using CD4 MicroBeads, human (# 130-045-101), were cultivated over night in RPMI with 5% AB serum without (A) or with (B) ionomycin (1 μ g/mL) and PMA (20 ng/mL). Cell culture supernatants were harvested and centrifuged to remove particulates. Diluted sample (1: 30) were analyzed using MACSplex Cytokine Reagents Kits, human for IL-2, IL-4, IL-5, IL-10, IL-17A, TNF- α , and IFN- γ in combination with MACSplex Cytokine 12 Standard, human and the MACSplex Cytokine Basic Kit. Two standard curves were carried out and the concentrations of seven analytes were determined using the MACSQuant Express Mode.

A: Unstimulated sample



B: stimulated sample



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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