Miltenyi Biotec

MACSPlex Cytokine Reagent Kits
mouse

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

Cytokine	Order no.
GM-CSF	130-109-702
IFN-γ	130-109-687
IL-2	130-109-686
IL-4	130-109-690
IL-5	130-109-692
IL-10	130-109-698
IL-12p70	130-109-696
IL-17A	130-109-685
IL-23	130-109-700
TNF-α	130-109-703

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, mouse

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-γ, IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-17A, IL-23, 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.

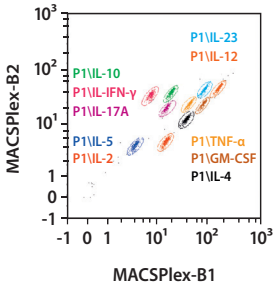


Figure 1: Detection of 10 MACSPlex Cytokine Capture Bead populations specific for mouse cytokine in a MACSPlex-B1 (FITC) versus MACSPlex-B2 (PE) dot plot.

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.

1.3 Applications

- MACSPlex Cytokine Reagents Kits have been designed for detection of up to seven analytes of choice in a single sample of the soluble mouse cytokines GM-CSF, IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-17A, IL-23, and TNF-α. The kits have been optimized for use with serum, plasma, and cell culture supernatants.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-005-028.03

1.4 Reagent requirements

- MACSPlex Cytokine 10 Standard, mouse (# 130-106-198)
- 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 25 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	2.999
IFN-γ	20.891
IL-2	2.144
IL-4	0.470
IL-5	0.379
IL-10	3.716
IL-12	4.725
IL-17A	1.864
IL-23	23.413
TNF-α	3.333

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	66.4	4.2	6.3
	Sample 2	369.4	10.2	2.8
	Sample 3	1950.9	68.6	3.5
IFN-γ	Sample 1	70.2	4.4	6.3
	Sample 2	354.5	10.5	3.0
	Sample 3	1895.0	69.9	3.7
IL-10	Sample 1	75.4	2.8	3.7
	Sample 2	365.9	7.4	2.0
	Sample 3	1964.1	48.5	2.5
IL-12	Sample 1	77.5	2.2	2.8
	Sample 2	377.3	3.3	0.9
	Sample 3	1985.2	8.3	0.4
IL-17A	Sample 1	74.7	4.1	5.5
	Sample 2	396.7	5.0	1.3
	Sample 3	1867.8	15.8	0.8
IL-2	Sample 1	63.3	8.6	13.5
	Sample 2	423.8	62.1	14.7
	Sample 3	2012.1	165.6	8.2
IL-23	Sample 1	80.9	6.0	7.5
	Sample 2	339.7	16.6	4.9
	Sample 3	1954.8	81.3	4.2
IL-4	Sample 1	68.7	3.2	4.7
	Sample 2	391.8	7.9	2.0
	Sample 3	1892.2	41.9	2.2
IL-5	Sample 1	69.9	2.6	3.7
	Sample 2	373.5	10.7	2.9
	Sample 3	1860.5	50.9	2.7
TNF-α	Sample 1	74.1	2.8	3.7
	Sample 2	372.8	11.6	3.1
	Sample 3	1988.1	37.5	1.9

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	396.0	32.5	8.2
	Sample 2	2019.5	244.0	12.1
IFN-γ	Sample 1	304.4	37.6	12.3
	Sample 2	1553.5	175.4	11.3
IL-10	Sample 1	360.4	31.1	8.6
	Sample 2	1695.1	279.0	16.5
IL-12	Sample 1	372.1	29.2	7.9
	Sample 2	1837.0	168.8	9.2
IL-17A	Sample 1	83.5	6.7	8.1
	Sample 2	409.8	42.7	10.4
IL-2	Sample 1	356.0	73.7	20.7
	Sample 2	1703.1	254.3	14.9
IL-23	Sample 1	73.7	12.8	17.4
	Sample 2	376.9	48.6	12.9
IL-4	Sample 1	86.6	8.6	9.9
	Sample 2	423.7	41.9	9.9
IL-5	Sample 1	84.7	7.3	8.6
	Sample 2	434.8	54.7	12.6
TNF-α	Sample 1	367.8	28.7	7.8
	Sample 2	1876.1	165.2	8.8

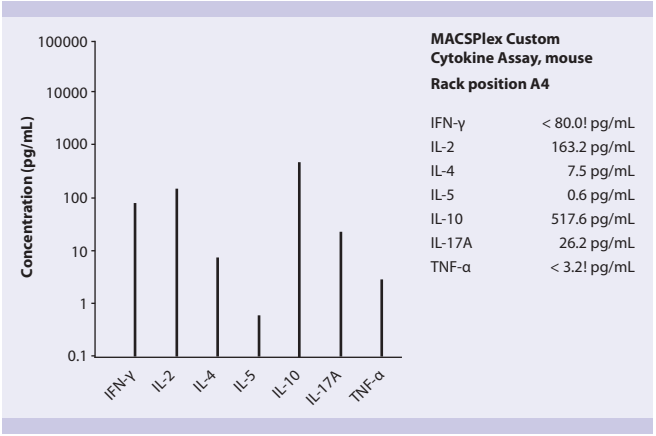
Specificity

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

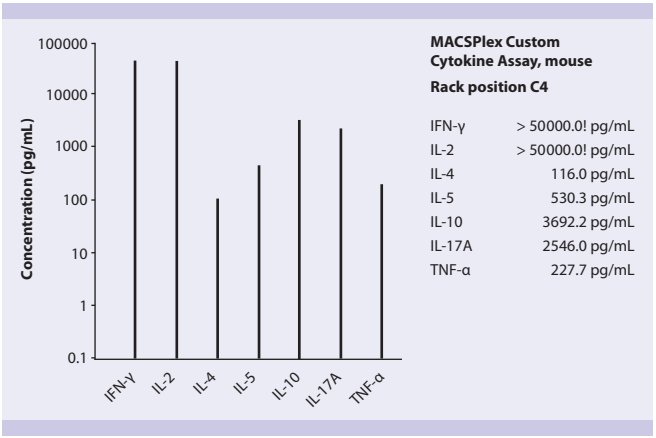
2.4 Exemplary results of the analysis using the Express Mode

Mouse spleen cells were cultivated over night in RPMI with 10% FBS without (A) or with (B) ionomycin (1 µg/mL) and PMA (10 ng/mL). Cell culture supernatants were harvested and centrifuged to remove particulates. Undiluted samples were analyzed using MACSPlex Cytokine Reagents Kits, mouse for IL-2, IL-4, IL-5, IL-10, IL-17A, TNF-α, and IFN-γ in combination with MACSPlex Cytokine 10 Standard, mouse 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



140-005-028.03

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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

MACSQuant and the Miltenyi Biotec logo, are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.