Powerful Tools for Macrophage/Microglia Research: New Multiplex Assay Panels Using Common Lab Flow Cytometers

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Abstract

Macrophages are among the first line of defenders and are also indispensable players in many other processes such as organ development, tissue turnover and regeneration, and tumor progression. Macrophages secrete an array of cytokines to effect their functions in host defense, tissue repair and immunoregulation. Pro-inflammatory macrophages (M1) produce cytokines, such as IL-1β, IL-6, IL-12p40, IL-12p70, IL-18, IL-23, CXCL10 (IP-10) and TNF-α, while anti-inflammatory and tissue repairing macrophages (M2) release a different set of factors, such as IL-1RA, IL-10, Arginase, CCL1 (I-309), CCL17 (TARC), CCL22 (MDC), CXCL1(GROα in human and KC in mouse), CXCL13 (BLC), TGF-β1 and other growth factors. In addition, the M2 macrophages are heterogenic and can be further divided into M2a, M2b, M2c and M2d subtypes. To correctly define the polarization status and identify the different types of macrophages is critical in understanding macrophage functions and related disease processes. We have developed new assay panels targeting human or mouse macrophage/microglia cells, using fluorescence–encoded beads that are suitable for use on general lab flow cytometers. These panels have been validated by detecting expected changes in biological samples. These high quality, low cost and easy-to-use panels provide an alternative multiplex solution to the biomedical research community.

Product	Cat. No.
LEGENDplex™ Human Macrophage/Microglia Panel (13-plex) with Filter Plate	740502
LEGENDplex™ Human Macrophage/Microglia Panel (13-plex) with V-bottom Plate	740503
LEGENDplex™ Mouse Macrophage/Microglia Panel (13-plex)	Coming Soon

Materials and Methods

1. Instrument and Settings

Flow Cytometer	Reporter Channel	Reporter Channel Emission	Beads Classification Channel	Classification Channel Emission	Compensation needed?
BD FACS Calibur™	blue & red	FL2	575 nm	FL4	660 nm
BD Accuri™ C6	blue & red	FL2	585 nm	FL4	675 nm
BD FACSCanto™, BD FACSCanto™ II	blue & red	PE	575 nm	APC	660 nm
BD LSR, BD LSRII, BD LSRFortessa™	blue & red	PE	575 nm	APC	660 nm
Gallios™	blue & red	PE	575 nm	APC	660 nm
CytoFLEX™	blue & red	PE	585 nm	APC	660 nm
NovoCyte™	blue & red	PE	572 nm	APC	660 nm
Attune™ NxT	blue & red	PE	574 nm	APC	670 nm

2. 96-well microtiter filter plates, V- or U-bottom plates, vacuum pump, filtration manifold and FACS tubes.
3. Capture antibody immobilized beads, biotinylated detection antibody cocktail, streptavidin-phycoerythrin (PE) conjugate and buffers.
4. Data analysis software and software dongle (provided free of charge).
5. **Biological Sample Preparation:**

   Human monocytes from healthy donors were isolated using Phicoll-PaqueTM (GE Healthcare) followed by positive selection with MojoSort™ Human CD14+ Monocytes Isolation Kit (BioLegend, San Diego). Cells were then differentiated and treated with the appropriate stimulations as described in the figure legend.

   Mouse macrophage cell line RAW264.7 or isolated splenocytes were treated with the appropriate stimulations as described in the figure legend.

   Cell culture supernatants were collected after 2 days .

Assay Protocol

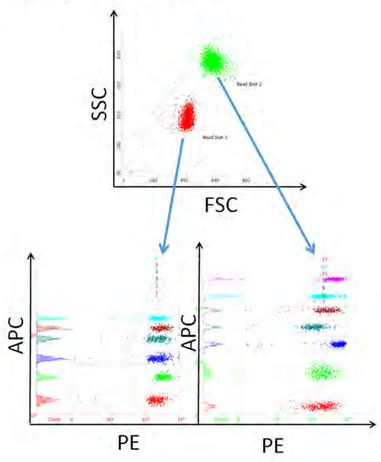
25 µL Matrix or Assay Buffer
25 µL Standard or samples
25 µL beads
↓ Shaking for 2h, RT
Vacuum and wash twice

25 µL Detection Antibody
↓ Shaking for 1h, RT
No vacuum, no wash

25 µL Streptavidin-Phycoerythrin
↓ Shaking for 30 min, RT
Vacuum and wash twice

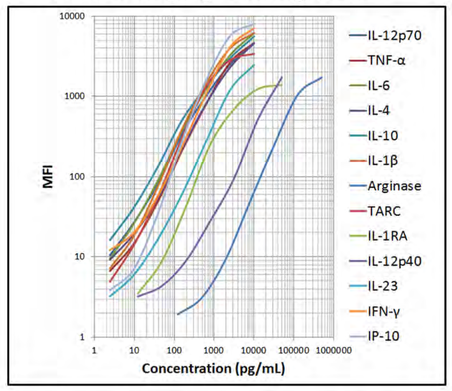
Read on a flow cytometer

Bead Classification

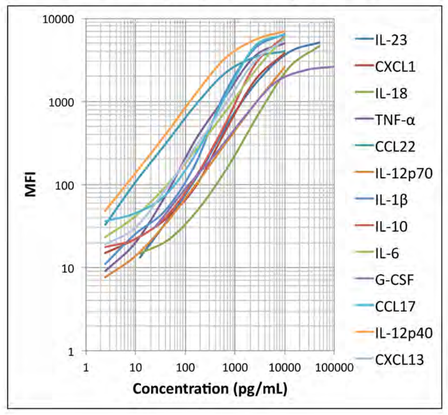


Representative Standard Curves

Human Macrophage / Microglia Panel



Mouse Macrophage / Microglia Panel



Panel Design and Sensitivities

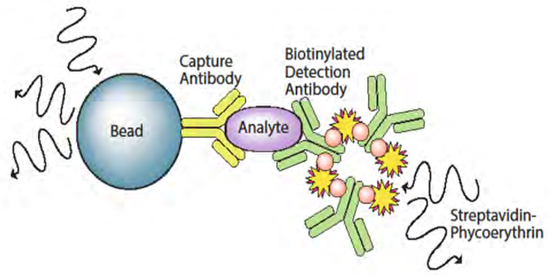
Human Macrophage / Microglia Panel

Macrophage/ Microglia Panel	M1 subpanel	M2 subpanel	Sensitivity (pg/mL)
IL-12p70	IL-12p70		1.3
TNF-α	TNF-α		1.3
IL-6	IL-6	IL-6	0.9
IL-4		IL-4	0.8
IL-10		IL-10	0.9
IL-1β	IL-1β		0.9
Arginase		Arginase	64.4
CCL17		CCL17	1.4
IL-1RA		IL-1RA	72
IL-12p40	IL-12p40		4.7
IL-23	IL-23		1.3
IFN-γ	IFN-γ		1.6
CXCL10	CXCL10		1.9

Mouse Macrophage / Microglia Panel

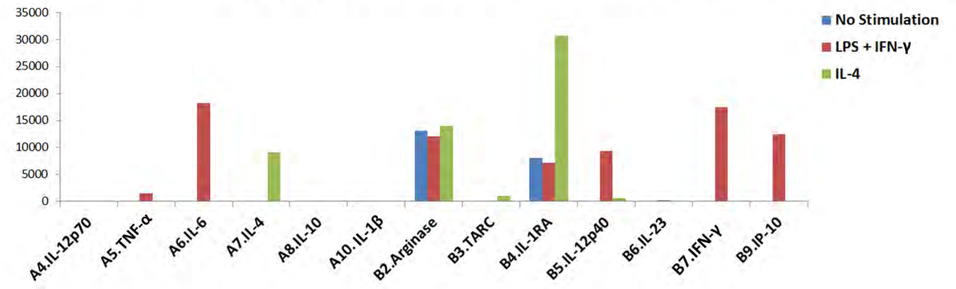
Macrophage/ Microglia Panel	M1 subpanel	M2 subpanel	Sensitivity (pg/mL)
IL-12p70	**IL-12p70**		**1.6**
TNF-α	**TNF-α**		**0.8**
IL-6	**IL-6**	**IL-6**	**1.0**
IL-18	**IL-18**		**4.2**
IL-10		**IL-10**	**2.2**
IL-1β	**IL-1β**		**1.0**
CCL22		**CCL22**	**0.6**
CCL17		**CCL17**	**1.8**
CXCL1	**CXCL1**		**2.3**
IL-12p40	**IL-12p40**		**0.8**
IL-23	**IL-23**		**5 .1**
G-CSF		**G-CSF**	**16**
CXCL13	**CXCL13**	**CXCL13**	**3.6**

Assay Principle



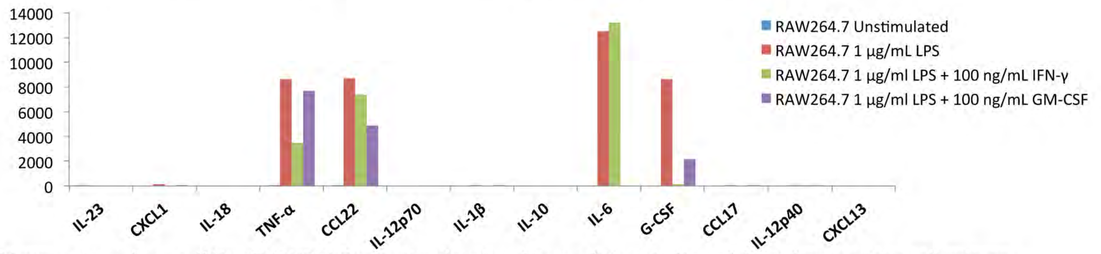
Biological Validation

Figure 1. Human M1/M2 macrophages polarized under different conditions (M1 vs. M2) display different cytokine profiles.



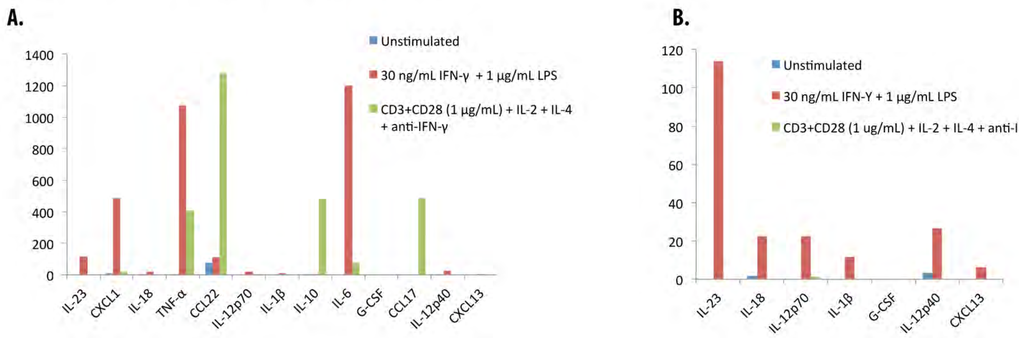
Monocytes were isolated from human PBMCs and differentiated for 5 days using GM-CSF (25 ng/mL). Differentiated monocytes were polarized under either M1 or M2 conditions for 48 h with LPS (100 ng/mL) + IFN-γ (25 ng/mL) or IL-4 (20 ng/mL). The adherent cells were washed, rested for 2 days in fresh media and re-stimulated with the same agents for another 48 h. The cell culture supernatant samples were collected and tested using the Human Macrophage/Microglia Panel.

Figure 2. Stimulated mouse macrophages cell line RAW264.7 displays a mixed cytokine profile.



Mouse macrophage cell line RAW264.7 was stimulated under conditions indicated for 3 days, and the cell culture supernatant samples were collected and tested using the Mouse Macrophage/Microglia Panel.

Figure3. LPS and IFN-γ stimulated mouse splenocytes produced a M1-like cytokine profile (high levels of IL-6, IL-23, CXCL1 and TNF-α, low levels of IL-18, IL-1β, IL-12p70 and IL-12p40 and CXCL13). Mouse splenocytes under Th2 polarizing conditions produced Th2-like (M2) cytokines (CCL22, IL-10, CCL17 and TNF-α).



Mouse splenocytes were isolated and stimulated under conditions indicated for 48 hours, and the cell culture supernatant samples were collected and tested using the Mouse Macrophage/Microglia Panel. In figure A, all 13 analytes are plotted together. In figure B, only low expression level analytes were plotted together.

Conclusions

We have developed bead-based multiplex assays for simultaneous quantification of 13 human or mouse macrophage/microglia related cytokines, providing powerful tools for biomedical research.

These assay panels offer high performance, low cost, easy-to-use, and no need for specialized instruments, with free user friendly software.

The utility of these multiplex assays was validated by using relevant biological samples.