affymetrix eBioscience

Macrophages and monocytes

Novel products for macrophage development in the immune system.

Featuring:

PrimeFlow™ RNA Assay

Intracellular markers: Functional phenotyping antibodies

- IDO (h/m)
- NOS2 (iNOS) (m)

Intracellular markers: Flow cytometry assay kits

- Total Reactive Oxygen Species (ROS) assay kits

Intracellular markers: Transcriptional control antibodies

- IRF5 (h)
- Phospho ERK1/2 (h/m)
- Phospho STAT3 (h/m)

Cell surface antibodies

- Mer (MerTK) (h)

MagniSort™: Cell enrichment kits and functional recombinant proteins

h=human, m=mouse

Macrophages constitute a very versatile population of phagocytic cells present in most tissues, albeit in different forms such as microglia, osteoclasts, and Kupffer cells. In response to cellular stresses, monocytes (macrophage precursors) are recruited into the tissue and are differentiated into macrophages. One popular classification divides activated macrophages into two polar categories: classically-activated macrophages (M1) and alternatively-activated macrophages (M2). Importantly, macrophage polarization is not permanent and can be altered following changes in their microenvironment.

M1 macrophages

M1 macrophages are pro-inflammatory and constitute a potent arm of the immune system deployed to fight infections. They are capable of having either direct (pathogen pattern recognition receptors) or indirect (Fc receptors, complement receptors) recognition of the pathogen. They can also produce reactive oxygen species (ROS) to help killing pathogens. M1 macrophages secrete pro-inflammatory cytokines and chemokines, attracting other types of immune cells and integrating and orchestrating the immune response. M1 activation is induced by IFNγ, TNFα, GM-CSF, LPS, and other TLR ligands. The most important transcription factors involved in this response are IRF5 and STAT1. The hallmarks of M1 activation are inducible nitric oxide synthase (iNOS) expression and high levels of IL-12 and low levels of IL-10 production. Other cytokines secreted by M1 cells include IFNγ, TNFα, IL-1, IL-6, IL-15, IL-18, and IL-23. M1 macrophages express high levels of major histocompatibility complex (MHC), co-stimulatory molecules, and FcγR.

M2 macrophages

M2 macrophages do not constitute a uniform population and often are further subdivided into M2a, M2b, and M2c categories. The common denominator of all three subpopulations is high IL-10 production accompanied by low production of IL-12. One of their signatures is production of enzyme Arginase-1 that depletes L-arginine, suppressing T cell responses, and depriving iNOS of its substrate. M2a macrophages are involved in the Th2 type immune response, e.g., against parasites, and are known to be profibrotic. They are induced by IL-4, IL-10, and IL-13, and are characterized by high surface expression of IL-4R and FcεR, Dectin-1, CD163, CD206, CD209, and other scavenger receptors. M2b macrophages are considered immunity-regulating and are induced by IL-1, LPS, and immune complexes. Besides IL-10, they also produce IL-1, IL-6, and TNFα. M2c macrophages are induced in the presence of IL-10 and TGFβ. They are often referred to as deactivated or anti-inflammatory, and are known to be involved in tissue repair and remodeling. They produce large amounts of IL-10 and TGFβ and express multiple receptors such as: CD163, CD206, RAGE, and other scavenger receptors. Multiple macrophage phenotypes cannot be readily classified into any of the groups, i.e., macrophages induced during various pathological conditions (e.g., tumor-associated macrophages).

In mouse, M1 macrophages can be characterized by expression of iNOS and M2 macrophages by Arginase-1, however, neither of these markers is expressed in these cells in humans.

Figure 1: Staining of Human Mer in Monocyte-derived Macrophages
Human monocyte-derived macrophages cultured in the presence of Human M-CSF Recombinant Protein (cat. no. 14-8789) and dexamethazone were stained with Anti-Human CD11b APC (cat. no. 17-0118) and Mouse IgG1 K Isotype Control PE (cat. no. 12-4714) (left) or Anti-Human Mer PE (cat. no. 12-9043) (right). Total viable cells, as determined by Fixable Viability Dye eFluor® 450 (cat. no. 65-0863), were used for analysis.

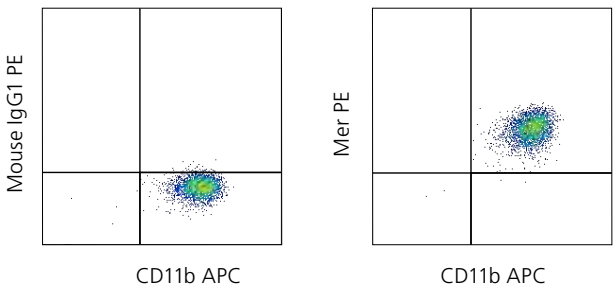
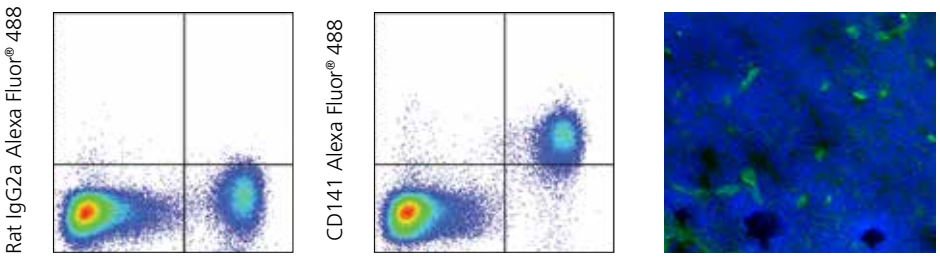


Figure 2: Flow cytometry and immunohistochemistry staining of Mouse CD141.
Staining of mouse resident peritoneal exudate cells with Anti-Mouse F4/80 Antigen APC (cat. no. 17-4801) and Rat IgG2α K Isotype Control Alexa Fluor® 488 (cat. no. 53-4321) (left) or Anti-Mouse CD141 Alexa Fluor® 488 (cat. no. 53-1411) (center). Total viable cells were used for analysis. Immunohistochemistry of frozen mouse lymph node using Anti-Mouse CD141 Alexa Fluor® 488. Nuclei are stained with DAPI (right).

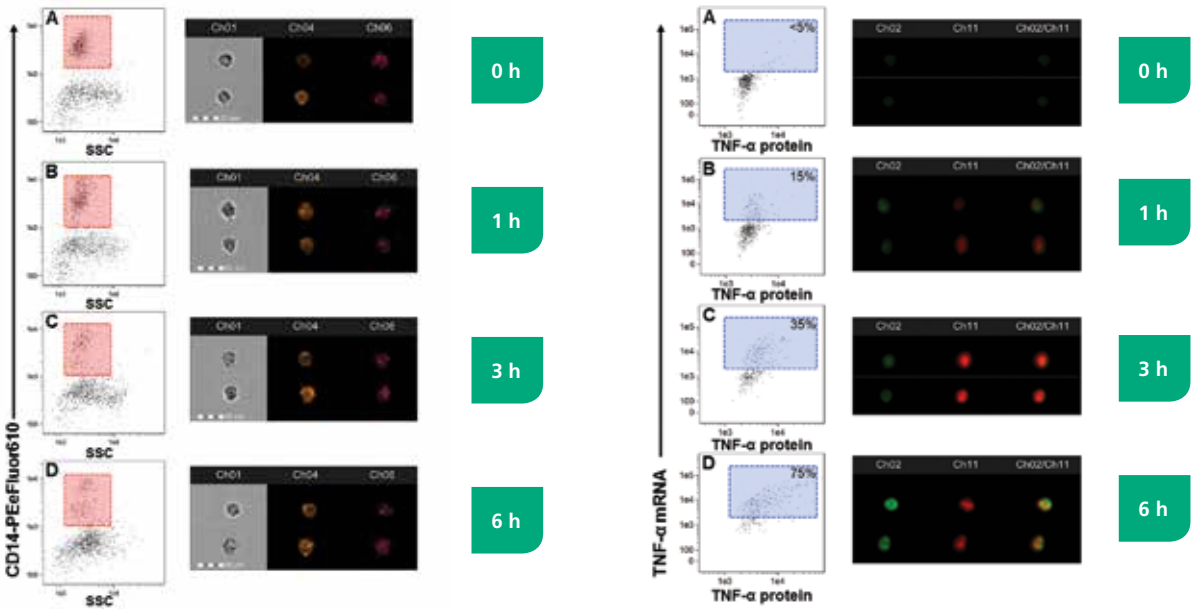


PrimeFlow™ RNA Assay

A new dimension in single-cell analysis

PrimeFlow™ RNA Assay reveals the dynamics of RNA and protein expression within individual cells, enabling their correlation in millions of single cells over time or in response to stimuli. This novel assay employs a proprietary fluorescent *in situ* hybridization (FISH) technique for simultaneous detection of up to three RNA transcripts in a single cell using a standard flow cytometer. RNA detection may be combined with intracellular and cell surface antibody staining to elevate the understanding of single-cell dynamics to a new dimension.

- See gene expression heterogeneity at the single-cell level
- Correlate RNA and protein level in the same cell
- Detect non-coding RNA in cellular subsets
- Evaluate viral RNA in infected cells
- Analyze mRNA expression levels when antibody selection is limited



Channel	Image
Ch01	Brightfield
Ch04	CD14-PE-eFluor® 610
Ch06	SSC
Ch02	TNFα Protein FITC
Ch11	TNFα RNA Alexa Fluor® 647
Ch02/Ch11	TNFα RNA/protein composite

Figure 3: Image-based flow cytometry to assess TNFα mRNA and protein production in monocytes following LPS stimulation
Normal human peripheral blood mononuclear cells were stimulated with LPS and Brefeldin A for 0-6 hours. Using PrimeFlow™ RNA Assay (cat. no. 88-18009), cells were fixed, permeabilized, and intracellularly stained with antibodies for CD14 (cat. no. 61-0149) and TNFα (cat. no. 11-7349). Next, cells underwent a series of hybridization steps to label mRNA for TNFα. All samples were acquired on a FlowSight® imaging flow cytometer. Viable CD14 cells were used for analysis.

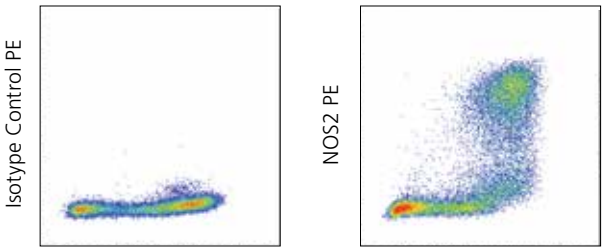
Data courtesy of Dr. Brian McFarlin, University of North Texas

Intracellular markers

Functional phenotyping antibodies

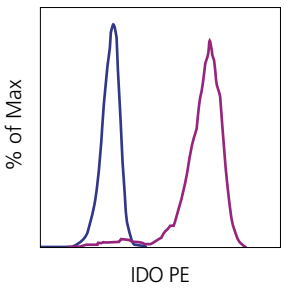
Functional Phenotyping Mouse Antibodies					
Target	Clone	Root SKU	Flow Cytometry	IHC	Neutralization
CCL2 (MCP-1)	2H5	7096	■	■	■
CCL3 (MIP-1α)	DNT3CC	7532	■		
IDO	mIDO-48	9473	■		
IFNγ	XMG1.2	7311	■		■
IL-1β pro-form	NJTEN3	7114	■		
IL-6	MP5-20F3	7061	■		■
IL-12/IL-23 p40	C17.8	7123	■		■
NOS2 (iNOS)	CXNFT	5920	■		
TNFα	MP6-XT22	7321	■	■	■

Figure 4: Anti-Mouse NOS2 staining in thioglycolate-elicited peritoneal exudate cells
Mouse thioglycolate-elicited peritoneal exudate cells were stimulated overnight with LPS, then surface-stained with Anti-Mouse CD11b PerCP-Cyanine5.5 (cat. no. 45-0112), followed by fixation and permeabilization with the Intracellular Fixation & Permeabilization Buffer Set (cat. no. 88-8824). The cells were then intracellularly stained with Rat IgG2a K Isotype Control PE (cat. no. 12-4321 [left]) or Anti-Mouse NOS2 PE (cat. no. 12-5920 [right]). Total viable cells, as determined by Fixable Viability Dye eFluor® 450 (cat. no. 65-0863), were used for analysis.



Functional Phenotyping Human Antibodies					
Target	Clone	Root SKU	Flow Cytometry	IHC	Neutralization
Arginase-1	sl6arg	9779		■	
CCL2 (MCP-1)	2H5	7096	■	■	■
CCL3 (MIP-1α)	CR3M	9706	■		
CCL4	FL34X3L	7540	■		
IDO	eyedio	9477	■		
	V1NC3IDO	9750		■	
IFNγ	4S.B3	7319	■		■
IL-1β	CRM56	7018	■		■
IL-6	MQ2-13A5	7069	■		■
IL-8	8CH	8088	■		
IL-12/IL-23 p40	C8.6	7129	■		■
TNFα	MAb11	7349	■		■

Figure 5: Anti-Human IDO staining in PBMCs
Surface staining of unstimulated (blue histogram) or overnight LPS-stimulated (purple histogram) normal human peripheral blood cells with Anti-Human CD3 PerCP-eFluor® 710 (cat. no. 46-0036) and Anti-Human CD11c FITC (cat. no. 11-0116) followed by staining with Fixable Viability Dye eFluor® 506 (cat. no. 65-0866). The cells were then intracellularly stained with Anti-Human IDO PE (cat. no. 12-9477), using the Intracellular Fixation & Permeabilization Buffer Set and protocol. Single, viable monocytes in the CD3-CD11c+ gate were used for analysis.



Flow cytometry assay kits

Reactive Oxygen Species (ROS) and Nitric Oxide (NO) Assays for Flow Cytometry		
Kit	SKU	Related Fluorochrome Channel
Total Reactive Oxygen Species (ROS) Assay - 520nm	88-5930	FITC

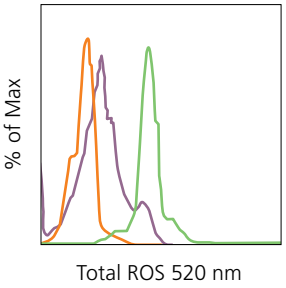


Figure 6: Flow cytometric staining of ROS
Staining of total reactive oxygen species (ROS) in unstimulated (orange histogram), 60-minute LPS-activated (purple histogram), or hydrogen peroxide–treated (green histogram) mouse peritoneal exudate cells using the Total Reactive Oxygen Species (ROS) Assay Kit 520 nm (cat. no. 88-5930). The assay was analyzed using the 488 nm (blue laser) in the FITC channel.

Transcriptional control

Transcriptional Control Antibodies					
Target	Clone	Root SKU	Crossreactivity	Flow Cytometry	IHC
IRF4	3E4	9858	Human/Mouse	■	
IRF5	ALYSCLN	9698	Human	■	
phospho ERK1/2 (T202/Y204)	MILAN8R	9109	Human/Mouse	■	■
phospho STAT1 (Y694)	KIKSI0803	9010	Human	■	
phospho STAT3 (Y705)	LUVNKLA	9033	Human/Mouse	■	
phospho STAT5 (Y694)	SRBCZX	9010	Human/Mouse	■	
phospho STAT6 (Y641)	CHI2S4N	9013	Human/Mouse	■	

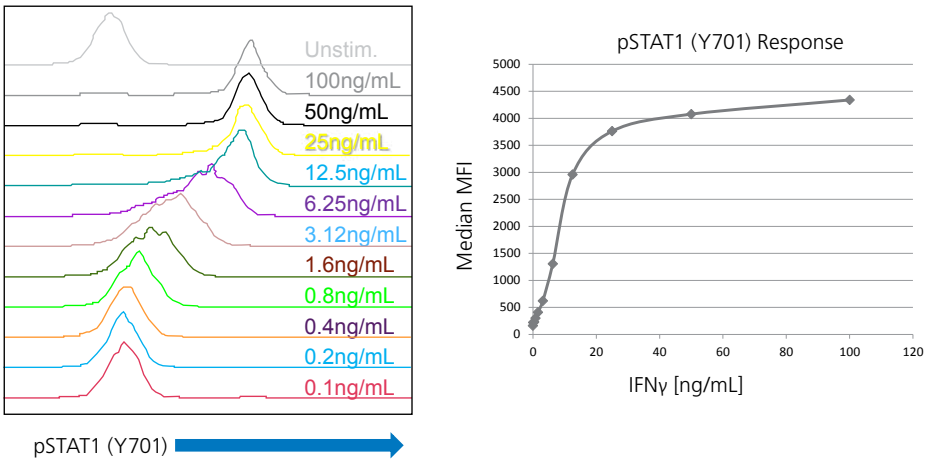


Figure 7: Staining of phospho STAT1 in U937 cells stimulated with interferon gamma
U937 cells were cultured in a 96-well plate and treated with various concentrations of Human IFNγ Recombinant Protein (cat. no. 14-8319) as indicated. Next U937 cells were intracellular stained with Anti- Human phospho-STAT1 eFluor®660 (cat. no. 50-9008). Medium fluorescent intensity (MFI) for each treatment concentration was plotted to generate dosage response curve.

Cell surface markers

Antibodies for flow cytometry, immunohistochemistry, and functional studies

Cell Surface Mouse Antibodies						Cell Surface Human Antibodies					
Target	Clone	Root SKU	Flow Cytometry	IHC/ ICC	Functional	Target	Clone	Root SKU	Flow Cytometry	IHC/ ICC	Functional
CD11a	HI111	0119	■	■	■	CD11a	M17/4	0111	■	■	■
CD11b	ICRF44	0118	■	■	■	CD11b	M1/70	0112	■	■	■
CD11c	3.9	0116	■	■		CD11c	N418	0114	■	■	
CD14	61D3	0149	■	■	■	CD14	Sa2-8	0141	■		
CD15	HI98	0159	■	■		CD16/CD32	93	0161	■		■
CD16	CB16	0168	■			CD32b	AT130-2	0321	■		
CD32	6C4	0329	■		■	CD63	NVG-2	0631	■		
CD33	WM53	0338	■	■		CD68	FA-11	0681	■	■	
CD63	H5C6	0639	■			CD80 (B7-1)	16-10A1	0801	■	■	■
CD64	10.1	0649	■	■		CD86 (B7-2)	FL1	0862	■	■	■
CD68	Y1/82A	0689	■	■		CD107b (LAMP-2)	ABL-93	1072	■	■	
CD80 (B7-1)	2D10.4	0809	■		■	CD115	AFS98	1152	■	■	■
CD86 (B7-2)	IT2.2	0869	■		■	CD141	LS17-9	1411	■	■	
CD107b (LAMP-2)	H4B4	1078	■			CD195 (CCR5)	HM-CCR (7A4)	1951	■		
CD115 (c-fms)	12-3A3-1B10	1159	■	■		CD197	4B12	1971	■		
CD162	FLEG	1629	■			CD207 (Langerin)	RMUL.2	2073	■	■	
CD163	GHI/61	1639	■			CD209 (DC-SIGN)	LWC06	2092	■		
CD195 (CCR5)	NP-6G4	1956	■			CD209b (SIGN-R1)	22D1	2093	■	■	■
CD197 (CCR7)	3D12	1979	■	■		CD274 (PD-L1)	MIH5	5982	■	■	■
CD206 (MMR)	19.2	2069	■			CD282 (TLR2)	T2.5	9024	■	■	■
CD209 (DC-SIGN)	eB-h209	2099	■			CD284 (TLR4)	UT41	9041	■		
CD274 (PD-L1)	MIH1	5983	■	■	■	F4/80	BM8	4801	■		■
CD282 (TLR2)	TL2.1	9922	■	■	■	FcεR	MAR-1	5898	■	■	■
CD284 (TLR4)	HTA125	9917	■	■	■	MHC Class I (H-2Db)	28-14-8	5999	■		
CD286 (TLR6)	hPer6	9069	■			MHC Class I (H-2Kb)	AF6-88.5.5.3	5958	■		
CD299 (DC-SIGN/L)	16E7	2999	■			MHC Class I (H-2Kd/H-2Dd)	34-1-2S	5998	■		
CX3CR1	2A9-1	6099	■			MHC Class I (H-2Kk)	AF3-12.1.3	5940	■		
FcεR	AER-37	5899	■			MHC Class Ib (H2-M3)	mAb 130	5230	■		
HLA-A2	BB7.2	9876	■			MHC Class II (1-A)	NIMR-4	5322	■		
HLA-A3	GAP.A3	5754	■			MHC Class II (I-A/I-E)	M5/114.15.2	5321	■	■	■
HLA-ABC	W6/32	9983	■	■		MHC Class II (I-Ab)	AF6-120.1	5320	■		
HLA-BC	B1.23.2	5935	■		■	MHC Class II (I-Ad)	AMS-32.1	5323	■		
HLA-DM	MaP.DM1	9703	■			MHC Class II (I-Ek)	14-4-4S	5980	■		
HLA-DQ	SK10	9881	■			MHC Classs I (H-2Kd)	SF1-1.1.1	5957	■		■
HLA-DR	LN3	9952	■	■		Thrombospondin-1	A6.1	9756		■	
HLA-E	3D12HLA-E	9953	■		■	TLR4/MD-2 Complex	MTS510	9924	■	■	■
HLA-G	87G	9957	■	■		Vimentin	V9	9897		■	
Macrophage marker	HAM56	6548		■							
Mer (MerTK)	HMER5DS	9043	■								
Thrombospondin-1	A6.1	9756		■							
Vimentin	V9	9897		■							

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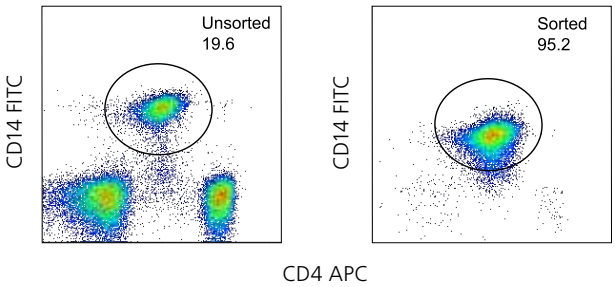
MagniSort™ cell enrichment kits and functional recombinant proteins

Isolate, differentiate, analyze

Whether cells are being activated *in vitro*, stained for and sorted by flow cytometry, or magnetically enriched for genetic analysis or other downstream experiments, the key to experimental success is reliability. MagniSort™ technology provides a simpler and faster cell enrichment method using a column-free magnetic separation platform. MagniSort products, combined with our broad antibody portfolio and immunology expertise, meet expectations for performance at a significant cost savings compared to column-based separation methods.

Features:

- Save time and money
- Treat cells gently with no passage through a column
- Eliminate cost and waste from disposable columns
- Process 2 x 10^9 cells per kit (two times that of other suppliers)



Fiture 8: Isolation of CD14+ cells from PBMCs
Normal human peripheral blood mononuclear cells were unsorted (left) or sorted with the MagniSort™ Human CD14 Positive Selection Kit (right) then stained with Anti-Human CD4 APC (cat. 17-0049) and Anti-Human CD14 FITC (cat. 11-0149). Total viable cells were used for analysis.

MagniSort™ Magnetic Cell Separation Kits			
Description	Part Number	Description	Part Number
MagniSort™ Human CD14 Positive Selection Kit	8802-6834	MagniSort™ Mouse F4/80 Positive Selection Kit	8802-6863
MagniSort™ Mouse CD11b Positive Selection Kit	8802-6860	MagniSort™ Streptavidin Negative Selection Beads	MSNB-6002
MagniSort™ Mouse CD11c Positive Selection Kit	8802-6861	MagniSort™ Streptavidin Positive Selection Beads	MSPB-6003

Functional Recombinant Proteins			
Description	Part Number	Description	Part Number
Lipopolysaccharide (LPS) Solution (500X)	00-4976	Mouse GM-CSF Recombinant Protein	14/34-8331
Human GM-CSF Recombinant Protein	14/34-8339	Mouse IFNγ Recombinant Protein	14/34-8311
Human IFNγ Recombinant Protein	14/34-8319	Mouse IL-4 Recombinant Protein	14/34-8041
Human IL-4 Recombinant Protein	14/34-8049	Mouse IL-10 Recombinant Protein	14/34-8109
Human IL-10 Recombinant Protein	14/34-8109	Mouse TGFβ 1 Recombinant Protein	14/34-8342
Human TGFβ 1 Recombinant Protein	14/34-8348	Mouse TNFα Recombinant Protein	14/34-8321
Human TNFα Recombinant Protein	14/34-8329		

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