TOOLS TO STREAMLINE YOUR MACROPHAGE RESEARCH

Macrophages are cells of the innate immune system, able to respond to infections or injury through phagocytosis as well as through the immunomodulatory cytokines they produce. Macrophages such as the "classically activated" M1 macrophages and "alternatively activated" M2 macrophages are cell types of great interest due to their role in immune regulation, tissue repair and tumor biology. STEMCELL Technologies can help you advance your research with a range of products to generate monocyte-derived macrophages for further downstream applications.

Generate Activated Macrophages Using ImmunoCult™-SF Macrophage Medium

Sourcing & Isolation | **Differentiation & Activation** | **Analysis**

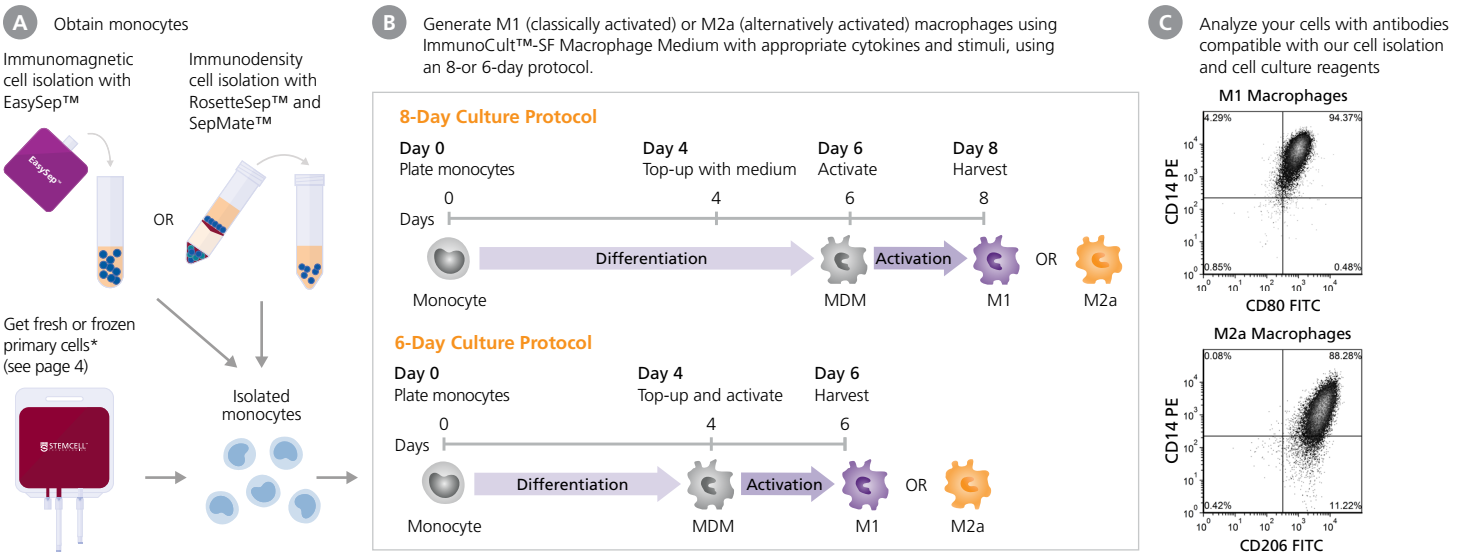

Figure 1. Integrated Workflow for the Generation of M1 or M2a Activated Macrophages using STEMCELL's Products.

(A) Isolate monocytes from fresh human whole blood or Leukopaks samples using EasySep™ negative selection kits or RosetteSep™ Enrichment Cocktails. (B) Generate monocyte-derived macrophages (MDM) from isolated monocytes by culturing the cells in ImmunoCult™-SF Macrophage Differentiation Medium (ImmunoCult™-SF Macrophage Medium (Catalog #10961) with added Human Recombinant M-CSF (Catalog #78057). With our 8-day protocol, top-up with fresh ImmunoCult™-SF Macrophage Differentiation Medium on Day-4 and drive specific macrophage activation using appropriate stimuli on Day-6 (IFN-γ+LPS for M1 activation and IL-4 for M2a activation). At Day-8 harvest fully mature M1 or M2a macrophages for use in downstream applications. With our 6-day protocol, macrophage activation can be done at the same time as the medium top-up step on Day-4 and harvested on Day-6. (C) Assess the phenotype and function of activated macrophages using STEMCELL's antibodies and ELISA kits.

*Fresh products currently available in the United States and Canada only. Please contact Technical Support for further information.

Why Use ImmunoCult™ to Generate Macrophages?

DEFINED FORMULATION. Medium is serum-free and does not require the addition of serum.

OPTIMIZED. Standardized formulation with pre-tested BSA to support macrophage differentiation and activation.

CONSISTENT. Obtain high yields of macrophages with the desired phenotype.

FLEXIBLE. Medium can be used with a variety of stimuli to achieve the desired macrophage subtype in an 8- or 6-day protocol.

Isolation | Differentiation & Activation | Analysis

1 Start Your Workflow by Isolating Monocytes Using EasySep™ or RosetteSep™

Use EasySep™ to Isolate Cells Immunomagnetically

Isolate monocytes without columns from virtually any sample source, including mononuclear cell suspensions and Leukopaks.

Use RosetteSep™ to Isolate Cells by Density Gradient Centrifugation

Isolate monocytes from whole blood during your standard density gradient centrifugation step.

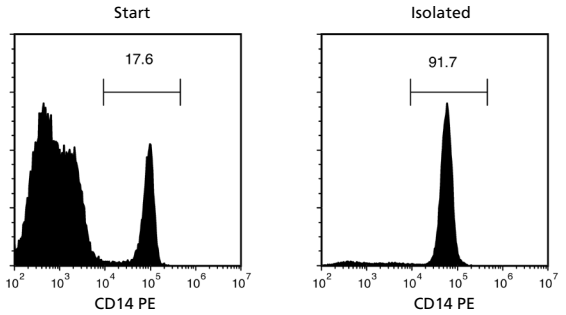

Figure 2. Highly Purified Monocytes Isolated with Negative Selection EasySep™ Human Monocyte Isolation Kit (Catalog # 19359)

Starting with mononuclear cells prepared from human whole peripheral blood, the monocyte cell content ($CD14^+CD45^+$) of the isolated fraction is typically 89.7 ± 3.4% (gated on CD45, mean ± SD, n=10).

Isolation | Differentiation & Activation | Analysis

2 Differentiate Monocytes into M1 and M2a Macrophages with the Desired Phenotype Using ImmunoCult™-SF Macrophage Medium

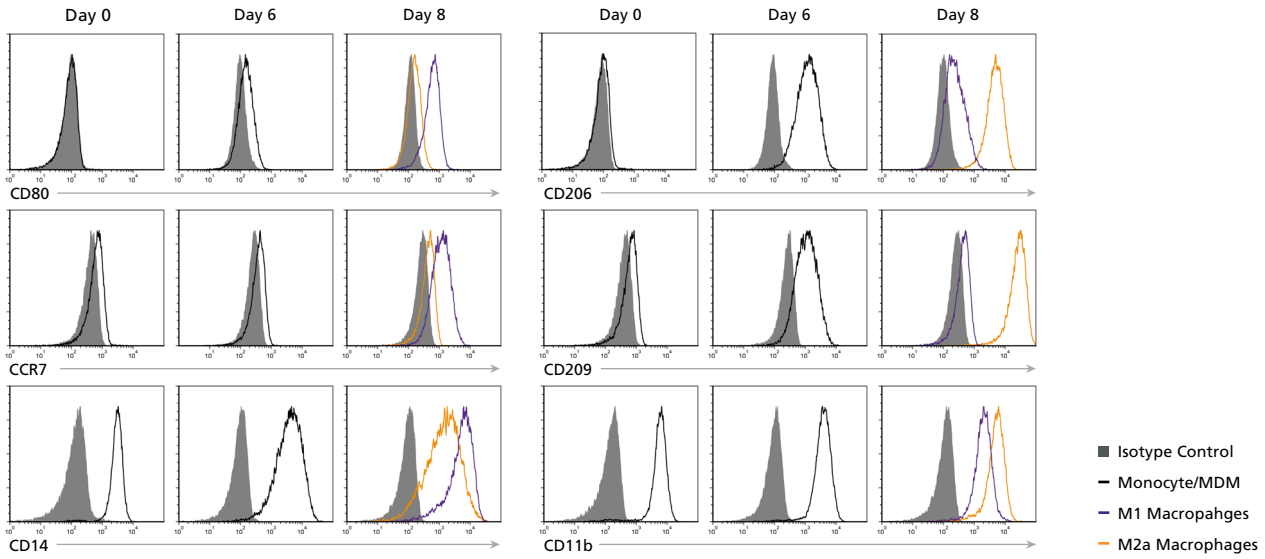

Figure 3. M1 and M2a Activated Macrophages Generated with ImmunoCult™-SF Macrophage Medium Show Desired Phenotype

EasySep™ isolated monocytes were cultured in ImmunoCult™-SF Macrophage Medium as described in Figure 1B using an 8-day protocol. Isolated monocytes (Day-0), monocyte-derived macrophages (MDM; Day-6) and M1 or M2a macrophages (Day-8) activated by IFN-γ+LPS, or IL-4 stimulation, respectively, were analyzed by flow cytometry for the expression of monocyte and macrophage markers compared to isotype control (Gray filled). At Day-8, M1 macrophages (Purple) expressed high levels of CD80 and CCR7 while M2a macrophages (Orange) upregulated CD206 and CD209 expression. Both M1 and M2a macrophages continue to express varying levels of CD14 and CD11b at the end of the culture period.

3 Obtain High Yields of Activated Macrophages with ImmunoCult™-SF

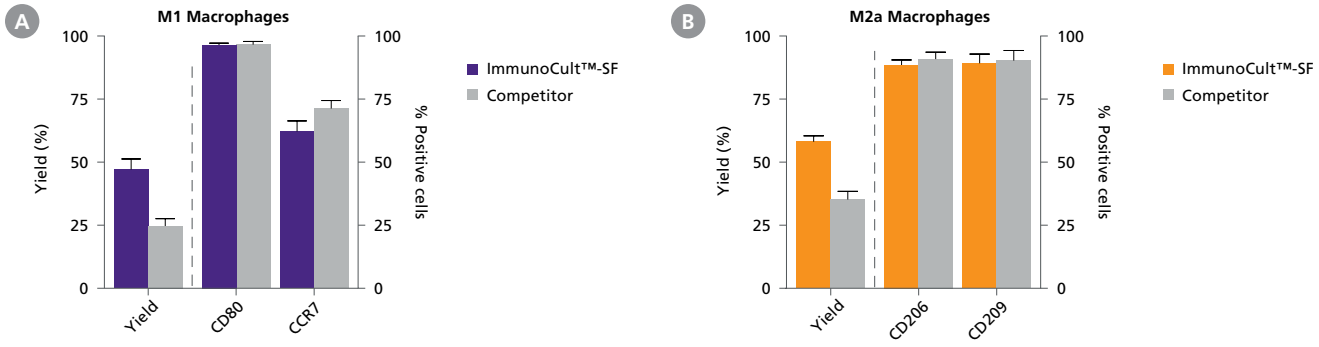

Figure 4. ImmunoCult™-SF Supports Greater M1 and M2a Macrophage Yields than Other Competitors' Serum-free Medium

Monocytes were cultured in ImmunoCult™-SF Macrophage Medium or a Competitor's serum-free macrophage medium and differentiated into macrophages using an 8-day protocol as shown in Figure 1B. At Day-8, macrophages were harvested, counted and analysed by flow cytometry to assess the expression of macrophage markers CD80, CCR7, CD206 or CD209. (A) M1 macrophages were $CD80^{+}CCR7^{+}$ whereas (B) M2a macrophages showed a $CD206^{+}CD209^{+}$ phenotype. Macrophage yields are expressed as a percentage of total viable cells at Day 8 relative to the count of initial monocytes at Day 0. Macrophage yields were significantly higher in ImmunoCult™-SF than in Competitor's serum-free medium ($P < 0.05$, paired t-test; mean ± SEM; n=18-19).

4 Assess the Function of Activated Macrophages Generated with ImmunoCult™-SF

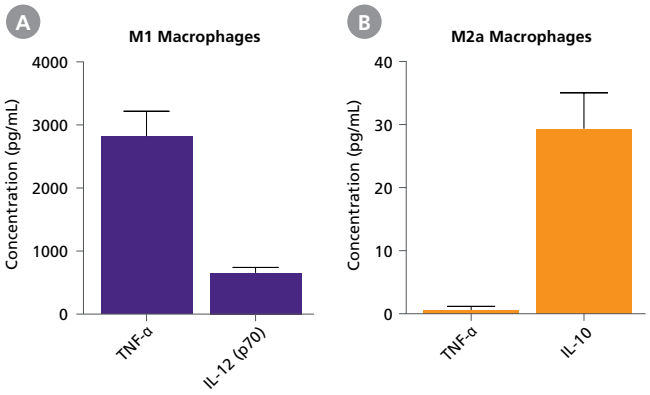

Figure 5. Activated Macrophages Generated with ImmunoCult™-SF Secrete the Appropriate Cytokines

Macrophages were generated with ImmunoCult™-SF Macrophage Medium and activated using IFN-γ+LPS (M1) or IL-4 (M2a) in an 8-day protocol. At Day-8, supernatants from M1 or M2a macrophage cultures were collected and the concentrations of TNF-α, IL-12 (p70) or IL-10 were determined by ELISA. (A) M1 macrophages secreted 2821 ± 396 pg/ml TNF-α (n=24) and 656 ± 86 pg/mL IL-12 (p70) (n=25). (B) M2a macrophages produced 29 ± 6 pg/mL IL-10 (n=21) and did not produce TNF-α (below limit of detection, n=20). Data represents the mean ± SEM.

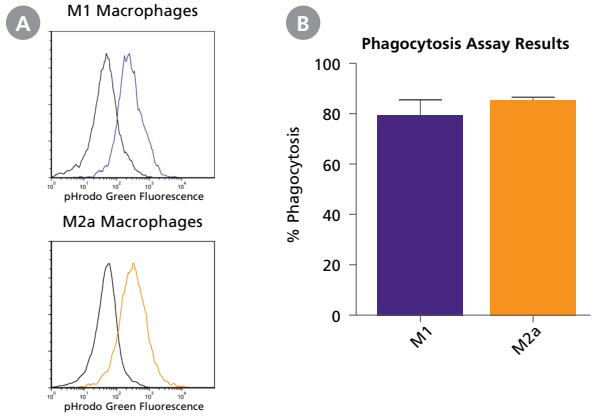

Figure 6. Activated Macrophages Generated with ImmunoCult™-SF Show Robust Phagocytic Activity

Phagocytic activity of M1 and M2a macrophages was assessed using PH-sensitive pHrodo® E.coli Green Bioparticles. M1 and M2a macrophages were generated according to the protocol described in Figure 1B and incubated with pHrodo E.coli Bioparticles for 1 hour at 37°C while negative control samples were incubated at 4°C. (A) M1 and M2a macrophages (purple and orange histograms, respectively) were then analysed by flow cytometry to detect the fluorescence from ingested E.coli particles as compared to their respective negative control (black histograms). (B) Average % phagocytosis from M1 and M2a macrophages are shown (n=3; Mean ± SEM).

Products for Your Macrophage Research Workflow

	Product	Catalog #	Description
Sourcing	**Primary Cells***		Choose from a wide range of fresh peripheral mononuclear cells and pre-isolated frozen monocytes that meet your donor specifications. **www.stemcell.com/PrimaryCells.** *Fresh products currently available in the United States and Canada (excluding Quebec). Certain cryopreserved products are only available in select territories. Please contact Product & Scientific Support (techsupport@stemcell.com) for additional information. †For optimal cell yield in this application, we recommend isolating monocytes from fresh blood products.
	Fresh Human Whole Peripheral Blood	70501 70504	
	Fresh Human Peripheral Blood Leukopak	70500	
	Frozen Human Peripheral Blood Mononuclear Cells (MNCs)	70025	
	Frozen Human Peripheral Blood Monocytes	70034	
Cell Isolation	**Immunomagnetic Cell Isolation**		Isolate monocytes from whole blood or PBMCs with fast, easy and column-free immunomagnetic or immunodensity cell separation platforms. • **FAST.** As little as a 12.5 minute cell isolation protocol • **SCALABLE.** Sample volumes from 0.1 – 40 mL • **GENTLE.** Column-free system, eliminates disposables. • **EASY WORKFLOW INTEGRATION.** Particles do not interfere with downstream applications **www.stemcell.com/CellSep** ‡For optimal cell yield in this application, we recommend isolating monocytes using negative selection products (e.g. Catalog #19359, Catalog #19058, Catalog #19669 and Catalog #15028).
	EasySep™ Human Monocyte Isolation Kit	19359	
	EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion	19058	
	EasySep™ Direct Human Monocyte Isolation Kit	19669	
	EasySep™ Human CD14 Positive Selection Kit II‡	17858	
	Immunodensity Cell Isolation		
	RosetteSep™ Human Monocyte Enrichment	15028	
	SepMate™-50	85450	
	Lymphoprep™ Density Gradient Medium	07801	
Differentiation & Activation	ImmunoCult™-SF Macrophage Medium	10961	Generate monocyte-derived macrophages using serum-free (SF) culture medium and optimized cytokines. • **DEFINED FORMULATION.** Medium is serum-free and does not require the addition of serum. • **OPTIMIZED.** Standardized formulation and protocols that support M1 and M2a macrophage differentiation and activation. • **CONSISTENT.** Results in high yield of cells with the desired phenotype. • **FLEXIBLE.** Medium can be used to generate various macrophage subtypes when activated with appropriate cytokines or stimuli.
	Recombinant Human M-CSF	78057	
	Recombinant Human IFN-γ	78020	
	Recombinant Human IL-4	78045	
	Recombinant Human GM-CSF	78015	
	Recombinant Human IL-10	78024	
	Lipopolysaccharide from E. coli (O55:B5)	100-1270	
Analysis	Anti-Human CD14 Antibody, Clone M5E2	60004	Analyze cells with antibodies that have been verified to work with our cell isolation reagents and cell culture media products. **www.stemcell.com/Antibodies**
	Anti-Human CD14 Antibody, Clone MoP9	60124	
	Anti-Human CD45 Antibody, Clone HI30	60018	
	Anti-Human CD32 Antibody, Clone IV.3 (FcR blocker)	60012	
	Human IL-12 (p70) ELISA Kit	02014 02015	Evaluate cell activation and/or differentiation by accurately quantifying cytokines of interest. **www.stemcell.com/ELISA**.
	Human IL-10 ELISA	02012 02013	

Other Products

Product	Catalog #	Description
ACCUTASE™	07920	Accutase™ is a solution of proteolytic and collagenolytic enzymes optimized for the detachment of M1 macrophages from adherent surfaces.
CryoStor® CS2	07932	Cryopreserve cells in cGMP-manufactured animal component-free and serum-free cell cryopreservation medium. • **QUALITY.** Manufactured under cGMP and ready for clinical applications. • **DEFINED FORMULATION.** Pre-formulated with 2%, 5%, or 10% USP-grade DMSO. • **HIGH CELL VIABILITY.** Designed to mitigate temperature-induced molecular stress responses during freezing and thawing. **www.stemcell.com/Cryopreservation**
CryoStor® CS5	07933	
CryoStor® CS10	07930	

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