DENDRITIC CELL RESEARCH

Isolate, Differentiate, and Analyze Dendritic Cells

Dendritic cells (DCs) are potent antigen presenting cells and key regulators of the immune response. These cells are of great interest for research in cancer immunotherapy, vaccines and infectious diseases. STEMCELL Technologies is committed to help you advance your research with a range of products to generate DCs for use in further downstream applications.

Generate Mature DCs using ImmunoCult™-ACF Dendritic Cell Medium and Supplements

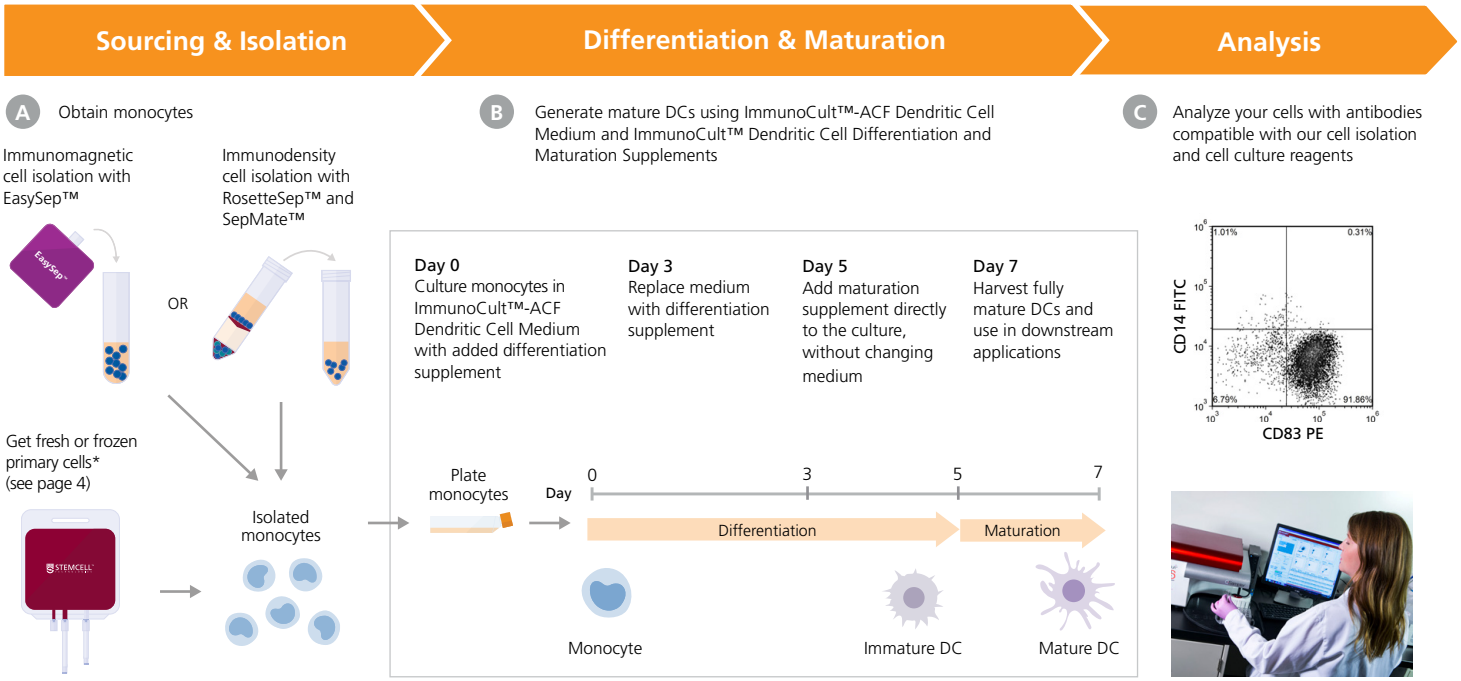

Figure 1. Integrated Workflow for the Generation of Mature DCs

(A) Isolate monocytes from fresh human whole blood or Leukopaks using EasySep™ negative selection kits or RosetteSep™ Enrichment Cocktails. To obtain cells from donors with your specific requirements get fresh* whole peripheral blood or Leukapheresis (Leukopak) preparations. (B) Generate mature DCs from isolated monocytes by culturing the cells in ImmunoCult™-ACF Dendritic Cell Medium (Catalog #10987) with added ImmunoCult™-ACF Dendritic Cell Differentiation Supplement (Catalog #10988) for three days. At day 3 remove the medium and add fresh medium with added differentiation supplement. At day 5, without changing the medium, add ImmunoCult™ Dendritic Cell Maturation Supplement (Catalog #10989) to the culture. At day 7 harvest fully mature DCs for use in downstream applications. (C) Assess the phenotype of mature DCs using STEMCELL's antibodies.

Why Use ImmunoCult™ to Generate DCs?

DEFINED FORMULATION. Medium and differentiation supplement are serum-free and animal component-free.

CONVENIENT. Ready-to-use and optimized formulation that supports DC differentiation and maturation.

RELIABLE. Obtain high yields of mature DCs with the desired phenotype and function.

FLEXIBLE. Medium and supplements can be used on their own or combined.

Sourcing & Isolation | Differentiation & Maturation | Analysis

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.

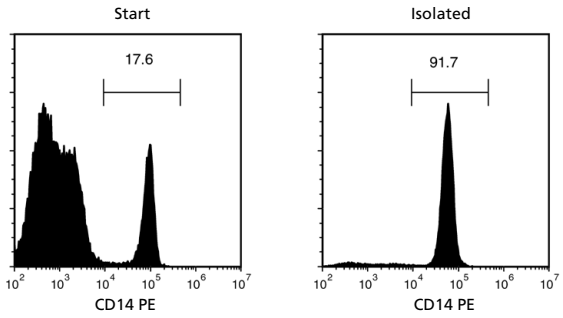

Figure 2. Highly Purified Monocytes Isolated with Negative Selection EasySep™ Human Monocyte Isolation Kit (Catalog # 19359)

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).

Sourcing & Isolation | Differentiation & Maturation | Analysis

Differentiate monocytes into DCs with ImmunoCult™-ACF Dendritic Cell Medium and Supplements

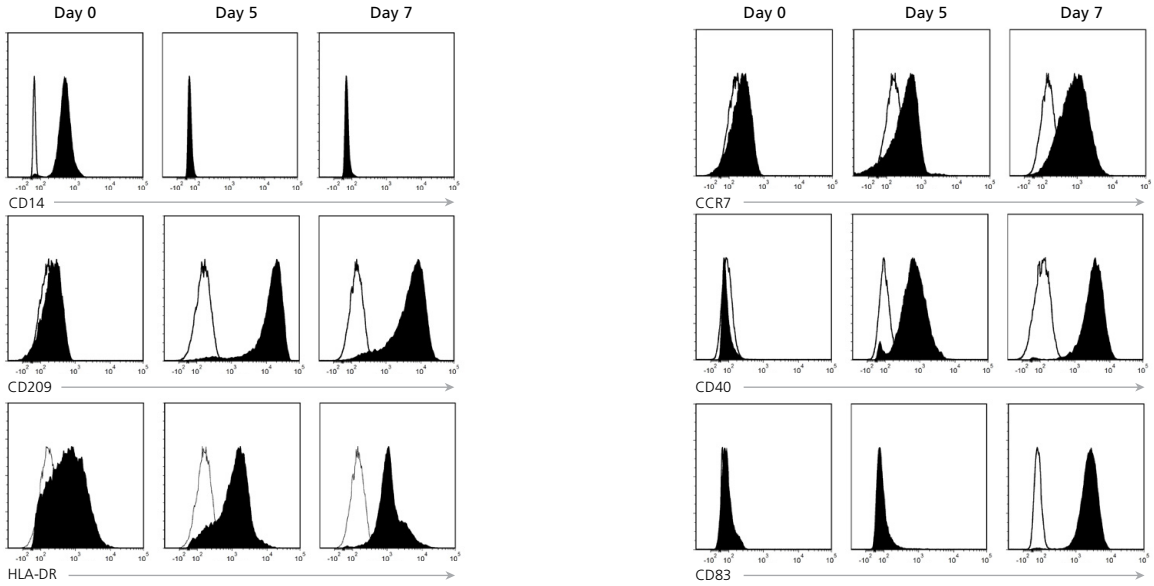

Figure 3. Monocytes Differentiate Into Mature DCs when Cultured in ImmunoCult™-ACF Dendritic Cell Medium with Supplements

On day 0, EasySep™ isolated monocytes were plated at 1 x 10^6 cells/mL and cultured as described in Figure 1B. Isolated monocytes (day 0), and cells harvested at day 5 (immature DCs) and day 7 (mature DCs) were analyzed by flow cytometry for the expression of monocyte and DC markers CD14, CD209 and HLA-DR and markers upregulated in mature DCs CCR7, CD40 and CD83.

Assess the phenotype of mature DCs generated with ImmunoCult™ using STEMCELL's antibodies

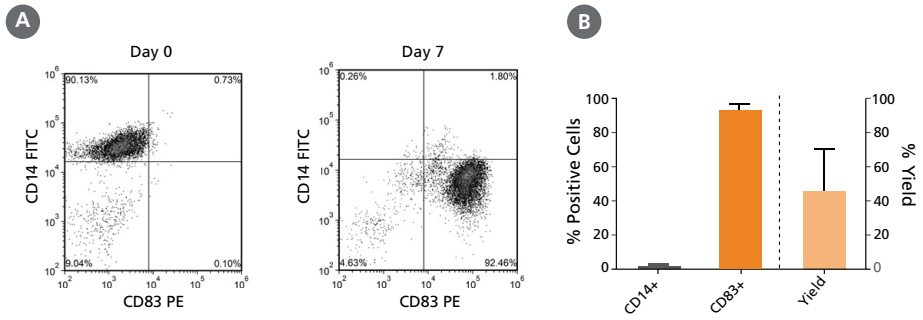

Figure 4. Mature DCs Generated with ImmunoCult™-ACF Dendritic Cell Medium and Supplements Show Desired Phenotype

EasySep™ isolated monocytes were cultured and differentiated into mature DCs as described in Figure 1B. (A) Representative flow cytometry plots of CD14 and CD83 expression in cells at day 0 (monocytes) and at day 7 (mature DCs). (B) The average percentage of CD14 and CD83 expression in cells at day 7 (mature DCs) was determined by flow cytometry. At day 7, a total of 93 ± 5% of the cells in culture expressed the mature DC marker CD83 and only 1 ± 1% of cells still expressed the monocyte marker CD14 (mean ± SD, n=39). Yield of mature DCs was determined by dividing the count of total viable cells at day 7 by the count of viable monocytes used at day 0. At day 7 the yield of viable mature DCs corresponded to 45 ± 25% (mean ± SD, n=39).

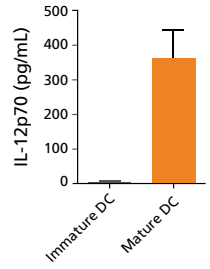

Figure 5. Mature DCs Generated with ImmunoCult™ Produce IL-12p70

DCs were generated with ImmunoCult™-ACF Dendritic Cell Medium and Supplements as described in Figure 1B. At day 5 cells were cultured with maturation supplement for 2 days (mature DCs) or without maturation supplement (immature DCs). Supernatant was collected at day 7 and IL-12p70 levels were determined by ELISA. Concentrations of IL-12p70 in supernatant of mature and immature DCs were 361 ± 81 and 5 ± 2 pg/mL, respectively (mean ± SEM, n=27).

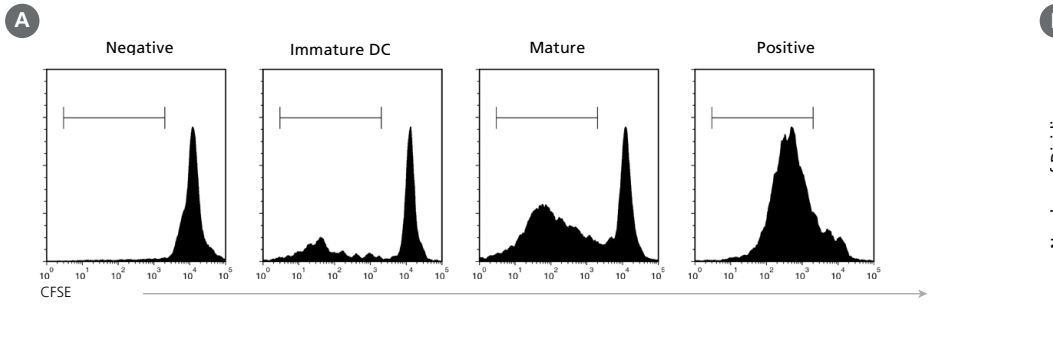

Figure 6. Mature DCs Generated with ImmunoCult™-ACF Dendritic Cell Medium and Supplements Induce T cell Proliferation

Immature DCs generated as described in Figure 1B, were loaded at day 5 with HLA Class I peptides derived from the human Cytomegalovirus, Epstein-Barr Virus and Influenza Virus (CEF peptide pool) and stimulated with maturation supplement (mature DCs) or left unstimulated (immature DCs) for 2 days. 1 x 10^5 autologous $CD8^+$ T cells isolated using EasySep™ were labeled with CFSE and cultured in Immunocult™-XF T Cell Expansion Medium alone (negative control), with peptide loaded immature or mature DCs at a DC:T cell ratio of 1:4 or 1:10 or with ImmunoCult™ Human CD3/CD28 T Cell Activator (positive control). After 5 - 7 days of culture the number of dividing T cells ($CD3^+CFSE^{lo}$) was determined by flow cytometry. (A) Representative histograms of CFSE dilution. (B) Average number of dividing T cells (mean ± SEM, n=4).

Products for Your DC Research Workflow

	Product	Catalog #	Description
Sourcing & Isolation	**Primary Cells***		Choose from a wide range of fresh peripheral mononuclear cells and pre-isolated frozen monocytes that meet your donor specifications. For a complete list of available primary cell products and full information on donor screening visit: **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 further information.
	Frozen Human Peripheral Blood Mononuclear Cells (MNCs)	70025	
	Frozen Human Peripheral Blood Monocytes	70034	
	Fresh Human Peripheral Blood Leukopak	70500	
	Fresh Human Whole Peripheral Blood	70501 70504	
	Immunomagnetic Cell Isolation		Isolate monocytes from whole blood or peripheral blood mononuclear cells (PBMCs) with fast, easy and column-free immunomagnetic or immunodensity cell separation platforms. • **FAST AND EASY.** Isolate monocytes from various sample sources, without columns or washes. • **SCALABLE.** Process sample volumes from 0.1 – 40 mL. • **HIGH PURITY.** Obtain highly purified and functional monocytes that are immediately ready for differentiation and maturation. **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 & Maturation	ImmunoCult™-ACF Dendritic Cell Medium (500mL)	10986	Generate monocyte-derived DCs using animal component-free (ACF) culture medium and supplements. • **CONVENIENT.** Ready-to-use and optimized formulation that supports DC differentiation and maturation. • **RELIABLE.** Obtain high yields of mature DCs with the desired phenotype and function. • **FLEXIBLE.** Medium and supplements can be used on their own or combined. §The kit includes Catalog #10987, Catalog #10988 and Catalog #10989.
	ImmunoCult™-ACF Dendritic Cell Medium (100mL)	10987	
	ImmunoCult™-ACF Dendritic Cell Differentiation Supplement (1mL)	10988	
	ImmunoCult™ Dendritic Cell Maturation Supplement (0.5mL)	10989	
	ImmunoCult™ Dendritic Cell Culture Kit§	10985	
Analysis	Anti-Human CD14 Antibody, Clone MoP9	60124	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 M5E2	60004	
	Anti-Human CD45 Antibody, Clone HI30	60018	
	Anti-Human CD83 Antibody, Clone HB15e	60107	

Other Products

Product	Catalog #	Description
CryoStor® CS2	07932	Cryopreserve cells in animal component-free and serum-free cell cryopreservation medium manufactured following current Good Manufacturing Practices (cGMP). • **READY-TO-USE.** Pre-formulated with 2%, 5% or 10% USP-grade DMSO. • **OPTIMIZED FORMULATION.** cGMP-manufactured, serum-free, and animal component-free media. • **HIGH CELL VIABILITY.** Designed to mitigate temperature-induced molecular stress during freezing and thawing.
CryoStor® CS5	07933	
CryoStor® CS10	07930	