TECHNICAL BULLETIN

# Generation of Monocytes from Human Pluripotent Stem Cells Using STEMdiff™ Medium and Supplements

## Background

Monocytes are essential components of the innate immune system that provide defense against pathogens or tumors due to their ability to differentiate to macrophages and dendritic cells. While monocytes can be isolated from peripheral blood, human pluripotent stem cells (hPSCs) offer another, potentially unlimited, source of monocytes. These hPSC-derived monocytes can be used in disease modeling, development of cell therapy applications, and research into basic biology.

STEMdiff™ Monocyte Kit facilitates the differentiation of hPSCs to monocytes under feeder-free and serum-free culture conditions.

## Differentiate hPSCs to Monocytes

STEMdiff™ Monocyte Kit is used to differentiate hPSCs to monocytes in a three-stage protocol as shown in Figure 1. In stage 1, Medium A differentiates hPSCs to the mesoderm lineage after 3 days of culture. In stage 2, the cells are cultured in Medium B for 4 days, promoting their specification to hematopoietic progenitor cells. During stage 3, Monocyte Differentiation Medium is used to promote the differentiation of progenitor cells to monocytes. These can be identified by the expression of CD14, as shown in Figure 2, and can be repeatedly harvested. STEMdiff™ Monocyte Kit is optimized for differentiation of multiple embryonic stem (ES) and induced pluripotent stem (iPS) cell lines maintained in TeSR™ PSC maintenance medium. After differentiation, hPSC-derived monocytes may be used in additional downstream assays.

### Why Use STEMdiff™ for Generating Monocytes?

**EFFICIENT.** Generate up to 7 million $CD14^+$ monocytes per plate in just 14 - 23 days.

**CONSISTENT.** Eliminate variation introduced by serum and feeder-cells by using serum- and feeder-free conditions.

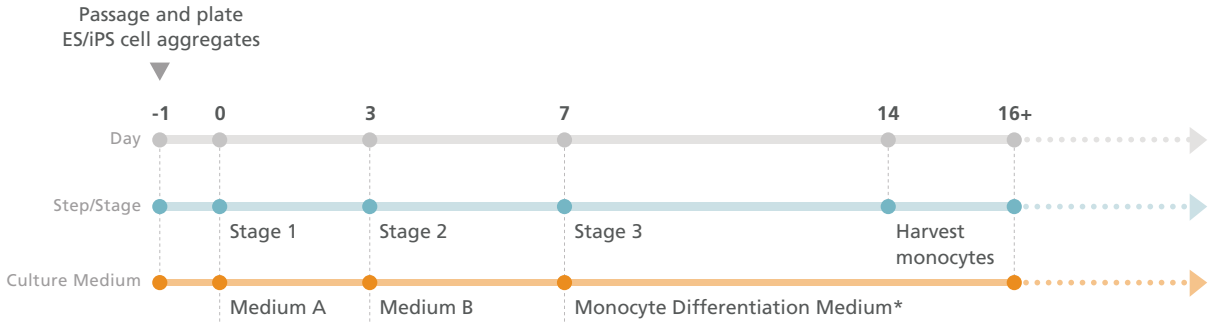
**EASY-TO-USE.** Produce monocytes in a simple monolayer culture for easier harvest of suspended cells.

**FLEXIBLE.** Achieve robust generation of monocytes across multiple ES and iPS cell lines.

| Product Name | Catalog # (Size) |
|---|---|
| STEMdiff™ Monocyte Kit | 05320 (1 Kit) |
| **Kit Components** | **Component # (Size)** |
| STEMdiff™ Hematopoietic Basal Medium | 05311 (120 mL) |
| STEMdiff™ Hematopoietic Supplement A (200X) | 05312 (225 µL) |
| STEMdiff™ Hematopoietic Supplement B (200X) | 05323 (225 µL) |
| STEMdiff™ Monocyte Differentiation Supplement (100X) | 05324 (3 x 1 mL) |
| StemSpan™ SFEM II | 09605 (3 x 100 mL) |

# Protocol for Differentiation of hPSCs to Monocytes

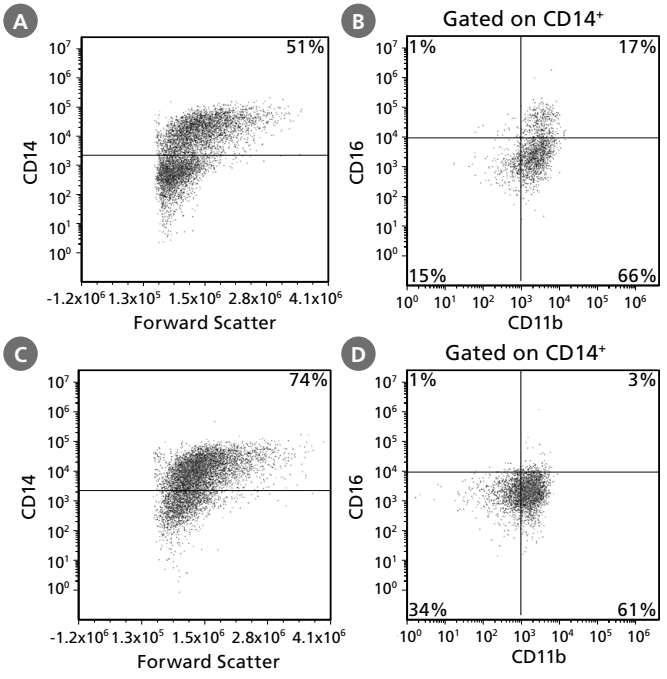
This protocol is designed to promote the differentiation of hPSCs to monocytes over 17 - 23 days of culture in a 2-dimensional (2D) culture system. The three stages — mesoderm formation (stage 1), hematopoietic specification (stage 2), and monocyte differentiation (stage 3) — are shown in Figure 1.




*Perform a full medium change every 2 - 3 days as needed.

**Figure 1.** Monocyte Differentiation Protocol

One day prior to differentiation, hPSC colonies are harvested and seeded as small aggregates (100 - 200 µm in diameter) at 10 - 20 aggregates/cm$^2$ in mTeSR™1, TeSR™-E8™, or mTeSR™ Plus. After one day, the medium is replaced with Medium A (STEMdiff™ Hematopoietic Basal Medium + Supplement A) to induce mesodermal specification (stage 1). On day 3, the medium is changed to Medium B (STEMdiff™ Hematopoietic Basal Medium + Supplement B) to promote hematopoietic specification (stage 2). On day 7, the medium is replaced with Monocyte Differentiation Medium (StemSpan™ SFEM II + STEMdiff™ Monocyte Differentiation Supplement) to promote the production of CD14$^+$ monocytes (stage 3). Monocyte Differentiation Medium is used for all medium changes for the remaining culture period. CD14$^+$ cells can be detected in suspension starting after day 14, and their frequency gradually increases until day 17 - 23. CD14$^+$ cells can be harvested directly from the culture supernatant during medium changes.



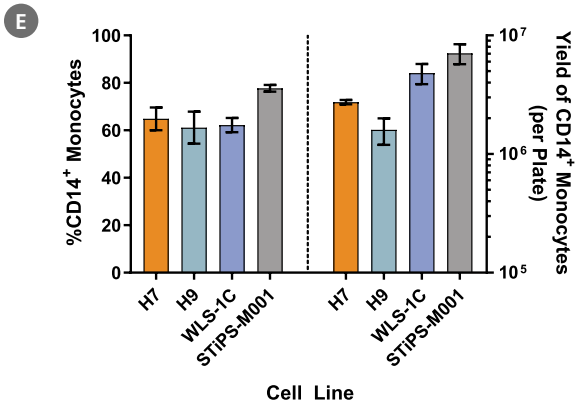




**Figure 2.** Robust and Efficient Generation of CD14$^+$ Monocytes Using STEMdiff™ Monocyte Kit

hPSCs were differentiated to monocytes using the 2D culture system described in Figure 1. Between days 17 and 23, cells were harvested every 2 - 3 days and analyzed by flow cytometry for CD14 expression. Representative flow cytometry plots are shown for (**A**,**B**) iPS (WLS-1C)-derived cells and (**C**,**D**) ES (H9)-derived cells. (**E**) The average frequency of viable CD14$^+$ monocytes at the peak harvest was 61 - 78%. The average yield of CD14$^+$ monocytes produced per 6-well plate at the peak harvest was between 1.6 x 10$^6$ and 7.1 x 10$^6$ cells. Data are shown as mean ± SEM (n = 3 - 14).

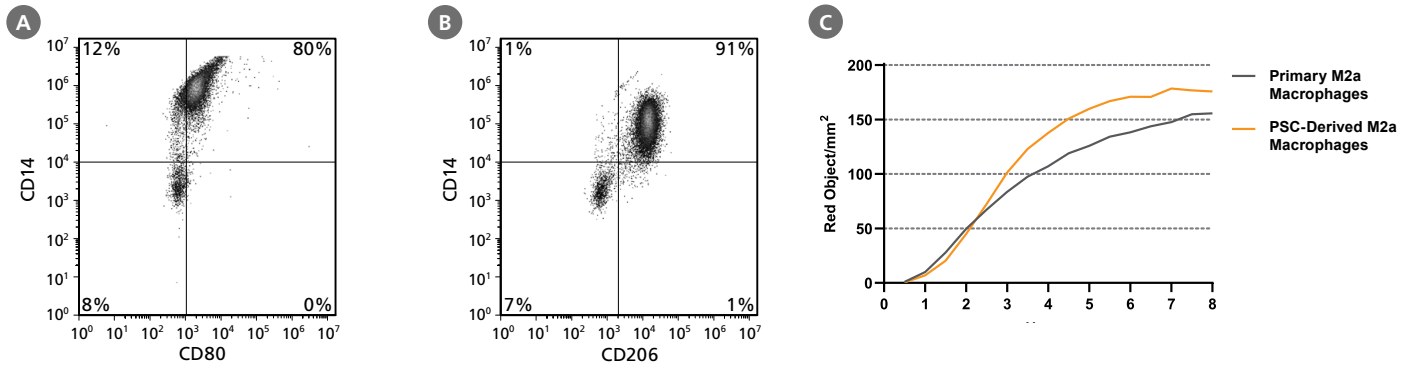



**Figure 3.** STEMdiff™ Monocyte Kit Generates Monocytes That Are Capable of Differentiation to Macrophages

hPSC-derived monocytes were harvested after 21 days of culture. These were then differentiated to macrophages using ImmunoCult™-SF Macrophage Medium with 100 ng/mL M-CSF for 4 days. Macrophages were then incubated for an additional 2 days with either 10 ng/mL of LPS and 50 ng/mL of IFN-γ, or 10 ng/mL IL-4, to become polarized to M1 or M2a macrophages, respectively. Representative flow cytometry plots of (**A**) M1 and (**B**) M2a macrophages produced from the WLS-1C iPS cell line are shown. (**C**) To measure phagocytosis, hPSC-derived M2a macrophages and peripheral blood (PB) monocyte-derived M2a macrophages (primary M2a macrophages), were incubated with pHrodo™ Red Zymosan A BioParticles® Conjugate and incubated at 37°C for 8 hours. Images were acquired using the IncuCyte® ZOOM every 30 minutes and analyzed for internalization of pHrodo™ Red Zymosan A BioParticles® (measured as red object/mm$^2$). hPSC-derived and primary M2a macrophages show similar phagocytic activity.

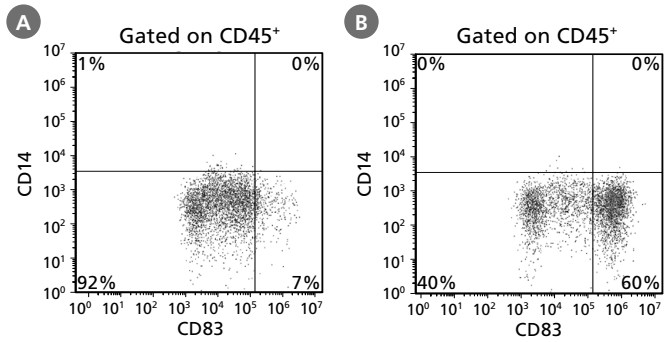



**Figure 4.** STEMdiff™ Monocyte Kit Generates Monocytes That Can Be Differentiated to Dendritic Cells

hPSCs were differentiated to monocytes, harvested after 21 days, and differentiated to dendritic cells using ImmunoCult™ Dendritic Cell Culture Kit. Half of the dendritic cells were harvested on day 7 and examined for CD14 and CD83 expression to identify $CD14^-CD83^{-/lo}$ immature dendritic cells. The remaining dendritic cells were activated for 2 days and assessed for the presence of $CD14^-CD83^+$ mature dendritic cells at day 7. Representative cultures initiated with ES (H9) cells are shown for production of (**A**) immature dendritic cells and (**B**) mature dendritic cells.

# Product Information

## Materials Required But Not Included

| Product Name | Size | Catalog # |
|---|---|---|
| mTeSR™1 | 500 mL Kit | 85850 |
| | 1 L Kit | 85857 |
| | 10 Kits | 85870 |
| | 25 Kits | 85875 |
| ***OR*** | | |
| TeSR™-E8™ | 1 Kit | 05990 |
| ***OR*** | | |
| mTeSR™ Plus | 1 Kit | 05825 |

| Product Name | Size | Catalog # |
|---|---|---|
| Gentle Cell Dissociation Reagent | 100 mL | 07174 |
| ***OR*** | | |
| ReLeSR™ | 100 mL | 05872 |
| ***OR*** | | |
| Dispase (1 U/mL) | 100 mL | 07923 |

| Product Name | Size | Catalog # |
|---|---|---|
| DMEM/F-12 with 15 mM HEPES | 500 mL | 36254 |
| 6-Well Flat-Bottom Plate, Tissue Culture-Treated | 50 Plates | 38015 |
| 96-Well Flat-Bottom Plate, Non-Treated | 50 Plates | 38044 |
| Human Recombinant IFN-γ | 100 µg | 78020 |
| Human Recombinant IL-4 | 100 µg | 78045 |

## Materials Recommended for Further Differentiation

| Product Name | Size | Catalog # |
|---|---|---|
| ImmunoCult™-SF Macrophage Medium | 250 mL | 10961 |
| Human Recombinant M-CSF | 100 µg | 78057 |

| Product Name | Size | Catalog # |
|---|---|---|
| ImmunoCult™ Dendritic Cell Culture Kit | 1 Kit | 10985 |
| Kit Components | | |
| ImmunoCult™-ACF Dendritic Cell Medium | 100 mL | 10987 |
| | 500 mL | 10986 |
| ImmunoCult™-ACF Dendritic Cell Differentiation Supplement | 1 mL | 10988 |
| ImmunoCult™ Dendritic Cell Maturation Supplement | 0.5 mL | 10989 |

## Recommended Antibodies for Analysis

| Product Name | Catalog # |
|---|---|
| Anti-Human CD14 Antibody, Clone M5E2 | 60004PE |
| Anti-Human CD16 Antibody, Clone 3G8 | 60041FI |
| Anti-Human CD11b Antibody, Clone ICRF44 | 60040AZ |
| Anti-Human CD83 Antibody, Clone HB15e | 60107PE |