STEMdiff™ Monocyte Kit

For differentiation of human ES and iPS cells to monocytes

Catalog #05320 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

STEMdiff™ Monocyte Kit includes serum-free media and supplements for the feeder-free differentiation of human embryonic stem (ES) and induced pluripotent stem (iPS) cells to monocytes expressing CD14.

The simple protocol is performed in 2D adherent cultures. During the first 3 days, Medium A induces cells toward mesoderm. For the subsequent 4 days, mesodermal cells are further differentiated toward the hematopoietic lineage using Medium B. At Day 7, the medium is changed to Monocyte Differentiation Medium, which facilitates the differentiation to monocytes. $CD14^+$ monocytes can be harvested directly from the culture supernatant starting as early as Day 14 and can be repeatedly harvested during the rest of the culture period. Peak $CD14^+$ frequency is typically 60 - 80%.

Product Information

The following components are sold as a complete kit (Catalog #05320); STEMdiff™ Monocyte Differentiation Supplement (Catalog #05324) and StemSpan™ SFEM II (Catalog #09605) are also available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Hematopoietic Basal Medium	05311	120 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Hematopoietic Supplement A (200X)	05312	225 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Hematopoietic Supplement B (200X)	05323	225 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Monocyte Differentiation Supplement (100X)	05324	3 x 1 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
StemSpan™ SFEM II	09605	3 x 100 mL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
6-well flat-bottom plate, tissue culture-treated	e.g. 38015
96-well flat-bottom plate, non-treated	e.g. 38044
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
Dispase (1 U/mL) OR Gentle Cell Dissociation Reagent OR ReLeSR™	07923 OR 100-0485 OR 100-0484
mTeSR™1 OR mTeSR™ Plus OR TeSR™-E8™	85850 OR 100-0276 OR 05990

Preparation of Media

Three different media are required for the monocyte differentiation protocol: **Medium A (Stage 1; Day 0 - 3)**, **Medium B (Stage 2; Day 3 - 7)**, and **Monocyte Differentiation Medium (Stage 3; Day 7+)**.

Prepare media as required according to section B of Directions for Use. Refer to Table 1 for medium components, volumes, and in-use storage and stability.

Use sterile technique to prepare Medium A (Basal Medium + Supplement A), Medium B (Basal Medium + Supplement B), and Monocyte Differentiation Medium (SFEM II + Monocyte Differentiation Supplement). Volumes indicated are for preparing 45 mL of Medium A, 45 mL of Medium B, and 100 mL of Monocyte Differentiation Medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™ Hematopoietic Basal Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 6 months, or aliquot and store at -20°C. After thawing aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-freeze. Do not exceed the shelf life of the medium.
2. Thaw StemSpan™ SFEM II at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Refer to the StemSpan™ SFEM II Product Information Sheet for complete thawing and handling instructions.
3. Thaw Supplement A, Supplement B, and Monocyte Differentiation Supplement at room temperature or at 2 - 8°C until just thawed. Mix thoroughly. If necessary, centrifuge for 30 seconds to remove liquid from cap.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately. Do not re-freeze.
4. Prepare media as indicated in Table 1. Mix thoroughly.

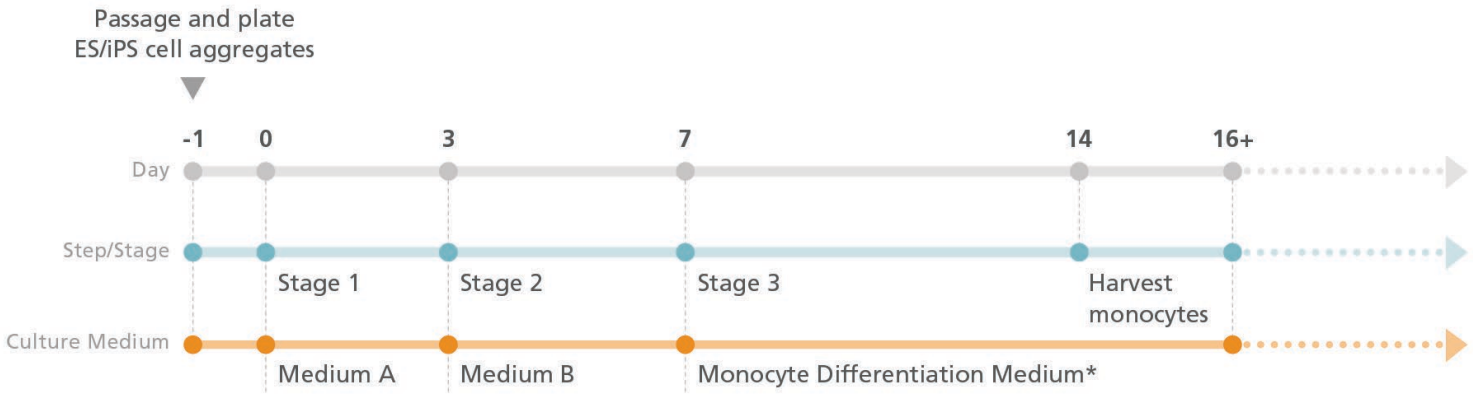
   NOTE: If not used immediately, store media as indicated in Table 1.

Table 1. Preparation of STEMdiff™ Monocyte Differentiation Media

COMPLETE MEDIUM	COMPONENT	VOLUME	IN-USE STORAGE AND STABILITY*
Medium A (45 mL)	STEMdiff™ Hematopoietic Basal Medium	45 mL	Store at 2 - 8°C for up to 4 weeks OR Store at -20°C for up to 6 months
	STEMdiff™ Hematopoietic Supplement A (200X)	225 µL	
Medium B (45 mL)	STEMdiff™ Hematopoietic Basal Medium	45 mL	Store at 2 - 8°C for up to 4 weeks OR Store at -20°C for up to 6 months
	STEMdiff™ Hematopoietic Supplement B (200X)	225 µL	
Monocyte Differentiation Medium (100 mL)	StemSpan™ SFEM II	100 mL	Store at 2 - 8°C for up to 4 weeks
	STEMdiff™ Monocyte Differentiation Supplement (100X)	1 mL	

*Do not exceed the shelf life of the components.

Protocol Diagram



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

Directions for Use

Please read all protocols before proceeding. Use sterile technique when performing the following protocols:

A. Passaging Cells as Aggregates and Differentiation Setup

B. Monocyte Differentiation

A. PASSAGING CELLS AS AGGREGATES AND DIFFERENTIATION SETUP

This protocol is for human ES and iPS cells cultured in either mTeSR™1, mTeSR™ Plus, or TeSR™-E8™. Use the medium with which the cells are routinely maintained and use whichever passaging reagent is preferred.

The setup instructions are for 6-well plates; indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly.

NOTE: For complete instructions on maintaining high-quality human ES and iPS cells and for coating plates with Corning® Matrigel®, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, or TeSR™-E8™, available at www.stemcell.com or contact us to request a copy.

Day -1

1. Coat cultureware with Corning® Matrigel® prior to passaging cells.
2. Passage human ES or iPS cells as aggregates of 100 - 200 µm in diameter using one of the following reagents:
   - Gentle Cell Dissociation Reagent: Passaging protocol as described in the Technical Manual for mTeSR™1, mTeSR™ Plus, or TeSR™-E8™
   - ReLeSR™: Passaging protocol as described in the Product Information Sheet (PIS) for ReLeSR™
   - Dispase (1 U/mL): Passaging protocol as described in the PIS for Dispase
3. Perform triplicate aggregate counts as described below to determine the average number of cell aggregates (≥ 50 µm in diameter) in a 5 µL sample:
   a. Aliquot 40 µL of DMEM/F-12 into 3 wells of a 96-well flat-bottom plate. Add 5 µL of aggregate mixture to each well.
   b. In each well, count aggregates that are ≥ 50 µm in diameter. Average the triplicate results and calculate the *Concentration of Cell Aggregates* (aggregates/µL).

   NOTE: For complete instructions on cell aggregate counting, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, or TeSR™-E8™, available at www.stemcell.com or contact us to request a copy.
4. Determine the *Number of Aggregates to Plate*. It is recommended to plate **100 - 200 aggregates/well** of a Matrigel®-coated 6-well flat-bottom plate (10 - 20 aggregates/cm^2) to achieve **40 - 100 colonies/well** (4 - 10 colonies/cm^2) adhered to the cultureware after 24 hours of incubation; however, multiple plating densities may need to be tested.
5. Calculate the *Plating Volume* of cell aggregate mixture for each condition in your experiment, as follows:

   Plating Volume (µL) = Number of Aggregates to Plate (step 4) ÷ *Concentration of Cell Aggregates* (step 3b)
6. Gently mix the cell aggregate mixture. Add the calculated *Plating Volume* (step 5) to each well of a 6-well plate coated with Matrigel® (prepared in step 1) and containing 2 mL of mTeSR™1, mTeSR™ Plus, or TeSR™-E8™.

   NOTE: If using split ratios, a range of 1 in 40 to 1 in 200 may be required depending on the confluence of the passaged well.
7. Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to distribute the cell aggregates. Do not disturb the plate for 24 hours.
8. Proceed to section B for differentiation.

B. MONOCYTE DIFFERENTIATION

The following instructions are for a 6-well plate; indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly.

NOTE: Throughout the protocol, warm all media to room temperature (15 - 25°C) before use. Do not leave media at room temperature for extended periods of time.

STAGE 1

Day 0

1. Confirm that **40 - 100 colonies/well** are adhered to the cultureware (i.e. 4 - 10 colonies per cm^2). Ensure to count all colonies, including tiny colonies with only a few cells.

   NOTE: To facilitate counting, aspirate medium and replace with fresh mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ (this will help to remove debris).

   CRITICAL: Do not proceed if cultures have < 40 colonies or > 100 colonies per well, as differentiation will be compromised.
2. Prepare Medium A (see Preparation of Media) required for Day 0 and Day 2 (i.e. total of 3.75 mL per well of a 6-well plate).
3. Aspirate medium from wells. Add 2.5 mL of Medium A per well. Incubate at 37°C for 2 days.

Day 2

4. Using a serological pipette, gently remove 1.25 mL of medium from each well and discard.
5. Gently add 1.25 mL of Medium A per well. Incubate at 37°C for 24 hours.

STAGE 2

Day 3

6. Prepare Medium B (see Preparation of Media) required for Day 3 and 5 (i.e. total of 3.75 mL per well of a 6-well plate).
7. Aspirate medium from wells. Gently add 2.5 mL of Medium B per well. Incubate at 37°C for 2 days.

Day 5

8. Using a serological pipette, gently remove 1.25 mL of medium from each well and discard.
9. Gently add 1.25 mL of Medium B per well. Incubate at 37°C for 2 days.

STAGE 3

Day 7

10. Prepare Monocyte Differentiation Medium (see Preparation of Media) required for Day 7+ (i.e. 2 mL per well of a 6-well plate on Day 7 and at each medium change).
11. Using a serological pipette, gently remove all medium from each well and discard.
12. Gently add 2 mL of Monocyte Differentiation Medium per well. Incubate at 37°C for 2 - 3 days.

Day 9 or 10

NOTE: At this point, floating cells can often be seen in culture, and they will increase in number for the remainder of the culture period.

13. Using a serological pipette, gently remove all medium from each well and discard.
14. Gently add 2 mL of Monocyte Differentiation Medium per well. Incubate at 37°C for 2 days.

Day 11 or 12

15. Using a serological pipette, gently remove all medium from each well and discard.
16. Gently add 2 mL of Monocyte Differentiation Medium per well. Incubate at 37°C for 2 days.

Day 14 - Harvest monocytes (optional)

At Day 14, monocytes can be harvested as described below. To continue culturing without harvesting, perform a full-medium change (steps 15 - 16) then proceed to step 21.

17. Using a serological pipette, gently remove all medium from each well; use this medium to rinse the well and loosen any partially adherent monocytes. Transfer medium containing monocytes to a collection tube.
18. Gently add 2 mL of Monocyte Differentiation Medium per well. Incubate at 37°C for 2 - 3 days.
19. Centrifuge collection tube containing monocytes (from step 17) at 300 x *g* for 5 minutes at room temperature. Remove and discard the supernatant.
20. Resuspend cell pellet in desired medium for analysis or downstream assays. The purity of the $CD14^+$ monocyte population can be determined by flow cytometry, if desired.

Day 16+

21. Every 2 - 3 days, either harvest monocytes (steps 17 - 20) or perform a full-medium change without harvesting (steps 15 - 16).

    NOTE: Maximum output of $CD14^+$ cells generally occurs between Day 17 and Day 22; this will vary between experiments.

Notes and Tips

For harvesting more monocytes at one time:

- When performing medium changes during the harvest period, increasing the volume of medium added (e.g. doubling the added volume to 4 mL per well for a 6-well plate) may increase yield in the next harvest.
- Instead of a medium change, perform a medium top-up. Add 2 mL of monocyte medium, without removing the supernatant. This allows monocytes to accumulate over 3 or 4 days instead of 2 days.
- Harvest monocytes during the highest production window (Day 17 - 22). The monocyte frequency may remain high after Day 22, but the cell yield is lower.