STEMdiff™ Erythroid Kit

For differentiation of human ES or iPS cells to erythroblasts

Catalog #100-0074 1 Kit

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Product Description

STEMdiff™ Erythroid Kit is designed for the serum-free and feeder-free differentiation of human embryonic stem (ES) and induced pluripotent stem (iPS) cells to erythroid progenitor cells (erythroblasts) expressing Glycophorin A and CD71. This simple 2D differentiation protocol is performed in two stages. During the first 10- to 12-day stage, STEMdiff™ Hematopoietic Supplement A and then Supplement E1 are added to basal medium to induce cells toward erythroid-biased hematopoietic progenitor cells. At the end of this stage, hematopoietic progenitor cells are easily harvested from the culture supernatant. During the second 14-day stage, cells are further differentiated to erythroid progenitor cells using Supplement E2 and StemSpan™ SFEM II (Day 10 - 24). The cells typically expand 241-fold +/- 190-fold (95% CI) during the 14-day erythroid differentiation stage, and the Day 24 cell population typically contains 74% +/- 5% (95 CI) CD71+GlyA+ erythroblasts. Cells generated using STEMdiff™ Erythroid Kit can be further matured into normoblasts and reticulocytes when moved to appropriate culture conditions for maturation.

STEMdiff™ Erythroid Kit has been optimized for differentiation of cells maintained in mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), and TeSR™-E8™ (Catalog #05990).

Product Information

The following components are sold as part of STEMdiff™ Erythroid Kit and are not 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™ Erythroid Supplement E1 (10X)	100-0075	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Erythroid Supplement E2 (10X)	100-0076	20 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
StemSpan™ SFEM II	09605	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 #
12-well tissue culture-treated plates	e.g. 200-0624
96-well flat-bottom plate	e.g. 38022
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
Gentle Cell Dissociation Reagent OR ReLeSR™ OR Dispase (1 U/mL)	100-0485 OR 100-0484 OR 07923
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
mTeSR™1 OR mTeSR™ Plus OR TeSR™-E8™	85850 OR 100-0276 OR 05990
Trypan Blue	07050

Preparation of Media

Three different media are required for the erythroid differentiation protocol: **Medium A (Day 0 - 3)**, **Medium E1 (Day 3 - 10)**, and **Medium E2 (Day 10 - 24)**.

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 E1 (Basal Medium + Supplement E1), and Medium E2 (SFEM II + Supplement E2). Volumes indicated are for preparing 45 mL of Medium A, 75 mL of Medium E1, and 65 mL of Medium E2. If preparing other volumes, adjust accordingly.

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

   NOTE: If not used immediately, store STEMdiff™ Hematopoietic Basal Medium at 2 - 8°C for up to 4 weeks, or aliquot and store at -20°C. For StemSpan™ SFEM II, aliquot and store at -20°C. After thawing aliquots, use immediately or store at 2 - 8°C for up to 4 weeks. Do not re-freeze. Do not exceed the shelf life of the media.

2. Thaw Supplements A, E1, and E2 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.

3. Prepare media as indicated in Table 1. Mix thoroughly.

   NOTE: If not used immediately, store complete medium at 2 - 8°C for up to 4 weeks, or at -20°C for up to 6 months.

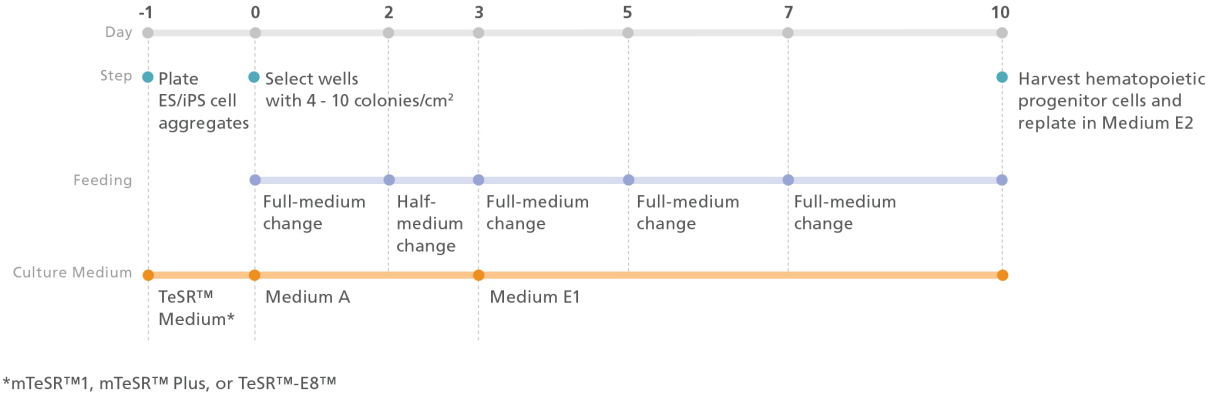
Table 1. Preparation of STEMdiff™ Erythroid Differentiation Media

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 E1 (75 mL)	STEMdiff™ Hematopoietic Basal Medium	67.5 mL	
	STEMdiff™ Erythroid Supplement E1 (10X)	7.5 mL	
Medium E2 (65 mL)	StemSpan™ SFEM II	58.5 mL	
	STEMdiff™ Erythroid Supplement E2 (10X)	6.5 mL	

*Do not exceed the shelf life of the components.

Protocol Diagrams

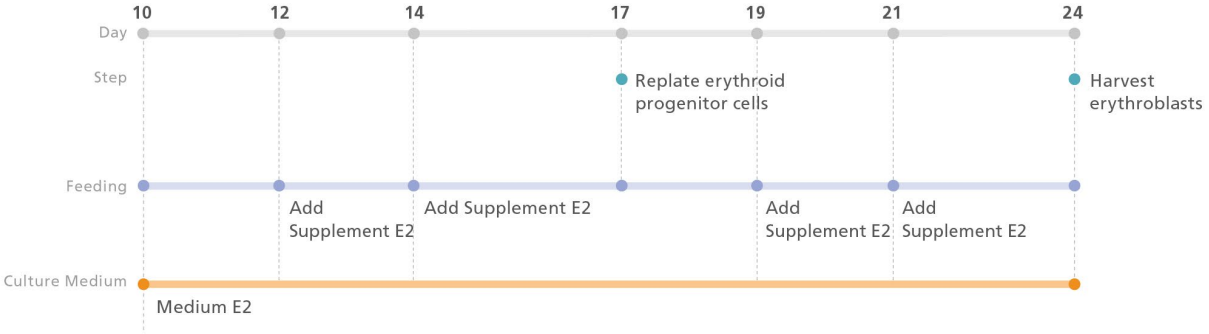
Day 0 - 10: Generation of Erythroid-Biased Hematopoietic Progenitor Cells



*mTeSR™1, mTeSR™ Plus, or TeSR™-E8™

On Day -1, harvest and seed human ES/iPS cell colonies as small aggregates in mTeSR™1, mTeSR™ Plus, or TeSR™-E8™. On Day 0 (after confirming the number of adhered colonies is within 4 - 10/cm^2), replace TeSR™ medium with Medium A. On Day 2, perform a half-medium change with fresh Medium A. On Day 3, change to Medium E1 and perform full-medium changes on Days 5 and 7. On Day 10, passage cells at 40,000 cells/mL into Medium E2.

Day 10 - 24: Erythroid Differentiation



Add Supplement E2 on Days 12 and 14. Passage cells on Day 17 at 167,000 cells/mL. Add Supplement E2 on Days 19 and 21. By Day 24, high yields of erythroblasts are typically generated.

Directions for Use

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

A. Passaging Aggregates and Differentiation Setup

B. Hematopoietic Differentiation

C. Erythroid Differentiation

D. Assessing Differentiation

E. Erythroid Maturation (Optional)

A. PASSAGING AGGREGATES AND DIFFERENTIATION SETUP

This protocol is for human ES or iPS cells cultured in 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 following instructions are for 12-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 Manuals for mTeSR™1, mTeSR™ Plus, or TeSR™-E8™, available at www.stemcell.com, or contact us to request a copy.

1. Coat a tissue culture-treated 12-well plate 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 **40 - 80 aggregates/well** (10 - 20 aggregates/cm^2) to achieve **16 - 40 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 12-well plate coated with Corning® Matrigel® (prepared in step 1) and containing 1 mL of either 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 hematopoietic differentiation.

B. HEMATOPOIETIC DIFFERENTIATION

The following instructions are for 12-well plates; 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.

Day 0

1. Confirm that **16 - 40 colonies/well** are adhered to the cultureware (4 - 10 colonies/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 < 16 colonies or > 40 colonies per well, as differentiation will be compromised.
2. Prepare Medium A (see Preparation of Media) required for Day 0 and Day 2 (a total of 1.5 mL per well of a 12-well plate).
3. Aspirate medium from wells. Add 1 mL of Medium A per well. Store remaining Medium A at 2 - 8°C until required.
4. Incubate at 37°C for 2 days.

Day 2

5. Using a serological pipette or a 1 mL pipette tip, gently remove 0.5 mL of medium from each well. Discard.
6. Gently add 0.5 mL of Medium A per well.
7. Incubate at 37°C for 24 hours.

Day 3

8. Prepare Medium E1 (see Preparation of Media) required for Days 3, 5, 7, and 10 (a total of 3.5 mL per well of a 12-well plate).
9. Aspirate medium from wells. Gently add 1 mL of Medium E1 per well. Store remaining Medium E1 at 2 - 8°C until required.
10. Incubate at 37°C for 2 days.

Day 5

11. Aspirate medium from wells. Gently add 1 mL of Medium E1 per well. Store remaining Medium E1 at 2 - 8°C until required.
12. Incubate at 37°C for 2 days.

Day 7

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

13. Aspirate medium from wells. Gently add 1 mL of Medium E1 per well. Store remaining Medium E1 at 2 - 8°C until required.
14. Incubate at 37°C for 3 days.

Day 10

NOTE: To increase cell yield, cells may be harvested on Day 12 instead of Day 10. However, the effect on erythroid differentiation needs to be determined, and the optimal harvest day is cell-line dependent.

If harvesting on Day 12, proceed to step 15; if harvesting on Day 10, proceed to step 16.

15. If harvesting on Day 12, perform the following steps on Day 10:
    a. Keep the plate flat and be careful not to disturb the floating cell population during this step. Using a serological pipette or a 1 mL pipette tip, gently remove 0.5 mL of medium from each well and discard.
    b. Gently add 0.5 mL of Medium E1 per well.
    c. Incubate at 37°C for 2 days.
    d. Proceed to step 16 to harvest supernatant cells.
16. Harvest supernatant cells as follows:
    a. Using a serological pipette or a 1 mL pipette tip, vigorously pipette the cells up and down in the well to break them up as needed (triturate).
    b. Transfer the cell suspension to a collection tube.
    c. Add 1 mL of StemSpan™ SFEM II to the well. Triturate vigorously in the well and add to the collection tube.
    d. Repeat step c.
    e. Centrifuge the tube at 300 x g for 5 minutes at room temperature (15 - 25°C).

f. Remove and discard the supernatant.
g. Resuspend cell pellet in StemSpan™ SFEM II and perform a viable cell count using Trypan Blue and a hemocytometer.

17. Proceed to section C for erythroid differentiation.

C. ERYTHROID DIFFERENTIATION

Day 10

18. Prepare Medium E2 (see Preparation of Media) required for Days 10 and 17 (a total of 2 mL per well of a 12-well plate).
19. Add 1 mL of Medium E2 per well of a tissue culture-treated 12-well plate. Store remaining Medium E2 at 2 - 8°C until required.
20. Add harvested cells from Step 16 to each well at 40,000 cells/mL. Incubate at 37°C for 2 days.

Day 12

21. Add 100 µL of Supplement E2 per well. Incubate at 37°C for 2 days.

Day 14

22. Add 100 µL of Supplement E2 per well. Incubate at 37°C for 3 days.

Day 17

23. Passage cells as follows:
    a. Using a serological pipette or a 1 mL pipette tip, gently pipette the cells up and down to wash the well.
    b. Transfer the cell suspension to a collection tube.
    c. Centrifuge the tube at 300 x g for 5 minutes at room temperature.
    d. Remove and discard the supernatant.
    e. Resuspend cell pellet in StemSpan™ SFEM II and perform a viable cell count using Trypan Blue and a hemocytometer.
    f. Add 1 mL Medium E2 per well of a 12-well plate.
    g. Add cells to each well at 167,000 cells/mL. Incubate at 37°C for 2 days.

Day 19

24. Add 100 µL of Supplement E2 per well. Incubate at 37°C for 2 days.

Day 21

25. Add 100 µL of Supplement E2 per well. Incubate at 37°C for 3 days.

Day 24

26. Harvest cells as follows:
    a. Using a serological pipette or a 1 mL pipette tip, gently pipette the cells up and down to wash the well.
    b. Transfer the cell suspension to a collection tube.
    c. Centrifuge the tube at 300 x g for 5 minutes at room temperature.
    d. Remove and discard the supernatant.
    e. Resuspend cell pellet in desired medium and perform a viable cell count for analysis or downstream assays. If desired, the purity of the CD71+GlyA+ erythroid cell population can be determined by flow cytometry.

D. ASSESSING DIFFERENTIATION

Assessing Erythroid Differentiation

The following antibodies are recommended for assessment of hPSC-derived erythroblasts by flow cytometry:

- Anti-Human CD71 (Transferrin Receptor) Antibody, Clone OKT9 (Catalog #60106)
- Anti-Human CD235a (Glycophorin A) Antibody, Clone 2B7, FITC (Catalog #60152FI)

E. ERYTHROID MATURATION (Optional)

Cells generated using STEMdiff™ Erythroid Kit have the capacity to mature into normoblasts and reticulocytes when they are moved to appropriate culture conditions for maturation. The following is a suggested maturation protocol:

1. Prepare maturation medium by adding the following to StemSpan™ SFEM II:
    - 3 U/mL Human Recombinant EPO (Catalog #78007)
    - 3% human AB serum

    Mix thoroughly. If not used immediately, store maturation medium at 2 - 8°C for up to 1 week.
2. Wash hPSC-derived erythroblasts (generated in section C) with either Iscove's MDM or StemSpan™ SFEM II (without added growth factors) to remove residual growth factors or other additives. Centrifuge at 200 x g for 5 - 10 minutes. Remove and discard the supernatant.

3. Resuspend cells at a concentration of 5 - 10 x 10^5 cells/mL in fresh maturation medium. Add 1 mL per well of a 12-well plate and incubate at 37°C for 3 - 4 days.
4. Add 1 mL of fresh maturation medium per well. Incubate at 37°C for 3 - 4 days.
5. After 7 days, harvest cells as described in section C step 26.