STEMdiff™ Neural Crest Differentiation Kit

Cell culture kit for establishment of hPSC-derived neural crest cells

Catalog #08610 1 Kit

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Product Description

STEMdiff™ Neural Crest Differentiation Medium is a serum-free medium for differentiation of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells to neural crest cells. These neural crest cells, which are characterized by neural crest markers such as SOX10 and CD271, can be differentiated to several downstream derivatives including chondrocytes, osteoblasts, and peripheral neurons. This medium is compatible with human ES and iPS cells maintained in either mTeSR™1 (Catalog #85850) or TeSR™-E8™ (Catalog #05990).

Product Information

The following components are sold as a complete kit (Catalog #08610) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Neural Induction Medium	05835	250 mL	Store at -20°C.	Stable for 12 months from date of manufacture on label.
STEMdiff™ Neural Crest Differentiation Supplement*	08611	0.5 mL	Store at -20°C.	Stable for 12 months from date of manufacture on label.

*Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	07174
Y-27632	72302
DMEM/F-12 with 15 mM HEPES	36254
Trypan Blue	07050
ACCUTASE™	07920

Preparation of STEMdiff™ Neural Crest Differentiation Medium

Use sterile techniques to prepare STEMdiff™ Neural Crest Differentiation Medium (STEMdiff™ Neural Induction Medium + STEMdiff™ Neural Crest Differentiation Supplement). The following example is for preparing 250 mL of complete medium. If preparing other volumes, adjust accordingly.

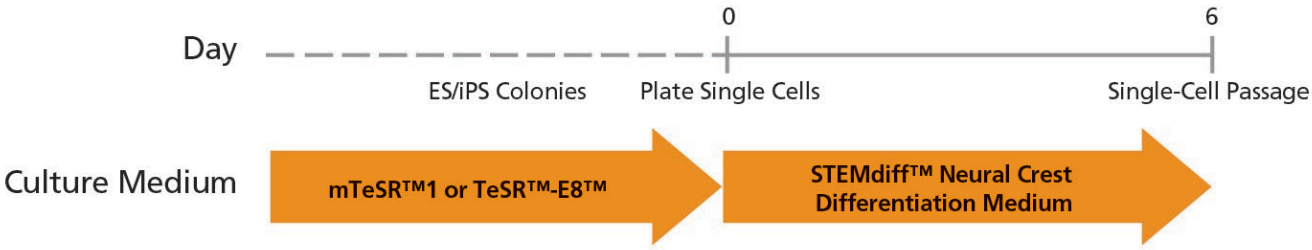
1. Thaw STEMdiff™ Neural Induction Medium and STEMdiff™ Neural Crest Differentiation Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the components. After thawing aliquots, use immediately. Do not re-freeze.

2. Add 0.5 mL of STEMdiff™ Neural Crest Differentiation Supplement to 250 mL of STEMdiff™ Neural Induction Medium. Mix thoroughly. Warm to room temperature (15 - 25°C) before use.

   NOTE: If not used immediately, store at 2 - 8°C for up to 2 weeks.

Protocol Diagram



Directions for Use

Please read the entire protocol before proceeding.

A. PLATING AND CULTURING SINGLE CELLS

The following instructions are for generating neural crest cells from human ES and iPS cells previously cultured in mTeSR™1 or TeSR™-E8™ in a 100 mm dish, and then plated into a single Matrigel®-coated well of a 6-well plate. If using other cultureware, adjust volumes accordingly.

Day 0

1. Coat one well of a 6-well plate with Corning® Matrigel®.

   NOTE: For complete instructions on coating plates with Corning® Matrigel®, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #28315), available at www.stemcell.com or contact us to request a copy.
2. Using a microscope, visually identify regions of differentiation in the human ES and iPS cell culture. Mark these using a felt tip or lens marker on the bottom of the 100 mm dish. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Wash the dish once with 5 - 10 mL of sterile D-PBS. Aspirate D-PBS.
4. Add 3 mL of Gentle Cell Dissociation Reagent. Incubate at 37°C for 8 - 10 minutes.

   NOTE: The incubation time may vary when using different cell lines or other non-enzymatic cell dissociation reagents, therefore dissociation should be monitored under the microscope until the optimal time is determined.
5. Dislodge cells by pipetting up and down 3 - 5 times using a pipettor. Using a 5 mL serological pipette, collect the cells into a 15 mL or 50 mL conical tube (e.g. Catalog #38009 or 38010). Break up any remaining cell aggregates into single cells by pipetting up and down.
6. Wash the dish with 10 mL of DMEM/F-12 and add to the tube containing the single-cell suspension.
7. Count viable cells using Trypan Blue and a hemocytometer.
8. Centrifuge cells at 300 x *g* for 5 - 10 minutes. Carefully aspirate the supernatant.
9. Resuspend cells in STEMdiff™ Neural Crest Differentiation Medium + 10 µM Y-27632 to obtain a final concentration of 1 x 10^6 cells/mL (i.e. 2 x 10^5 cells/cm^2).
10. Using a serological pipette or aspiration, gently remove the excess Matrigel® solution from the plate prepared in step 1. Ensure that the coated surface is not scratched.
11. Add 2 mL of cell suspension (2 x 10^6 cells/well) to one coated well of the 6-well plate.
12. 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 cells across the surface of the well. Incubate at 37°C for 24 hours.

Days 1 - 6

13. Perform a daily full-medium change with STEMdiff™ Neural Crest Differentiation Medium (Y-27632 is no longer required). Use 2 mL/well for a 6-well plate.
14. After reaching 95 - 100% confluency (approximately day 6), cells are ready for passaging. Proceed to section B.

B. PASSAGING NEURAL CREST CELLS

The following are instructions for passaging cells from one well of a 6-well plate to a Matrigel®-coated well of a new 6-well plate. If using other cultureware, adjust volumes accordingly.

1. Coat one well of a 6-well plate with Corning® Matrigel®.
2. Prepare sufficient volumes of STEMdiff™ Neural Crest Differentiation Medium, DMEM/F-12, and ACCUTASE™.
3. Aspirate medium from cells to be passaged.

OPTIONAL: Wash cells to be passaged with 1 mL of DMEM/F-12.

4. Add 1 mL of ACCUTASE™ to cells. Incubate at 37°C for 5 - 10 minutes.
5. Dislodge remaining attached cells by pipetting up and down using a 1 mL pipettor.
6. Add 5 mL of DMEM/F-12 and transfer the cell suspension to a 15 mL conical tube.
7. Centrifuge at 300 x *g* for 5 minutes. Carefully aspirate the supernatant.
8. Add 1 mL STEMdiff™ Neural Crest Differentiation Medium.
9. Count viable cells using Trypan Blue and a hemocytometer.
10. Using a serological pipette or aspiration, gently remove the excess Matrigel® solution from the plate prepared in step 1. Ensure that the coated surface is not scratched.
11. Add 2 mL STEMdiff™ Neural Crest Differentiation Medium to one coated well of the 6-well plate, and add cells at desired density.
12. 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 cells across the surface of the well.

    NOTE: For information on continued culture and passaging of neural crest cells, contact us at techsupport@stemcell.com.

Assessment of Neural Crest Differentiation

For evaluating neural crest induction, the optimal timepoint for assessment is between days 5 - 7. Antibodies for neural crest markers such as anti-SOX10 or anti-CD271 can be used alone or in combination to evaluate the phenotype of neural crest cells during differentiation. Cells should retain marker expression throughout at least two passages. To determine the presence of potentially contaminating neural cells, anti-PAX6 or anti-SOX1 antibodies can be used. Results may vary depending on cell line used.