

STEMdiff™ Sensory Neuron Differentiation Kit

STEMdiff™ Sensory Neuron Maturation Kit



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

Catalog #100-0341 1 Kit
 Catalog #100-0684 1 Kit

Product Description

The serum-free STEMdiff™ Sensory Neuron System comprises STEMdiff™ Sensory Neuron Differentiation Kit (Catalog #100-0341) and STEMdiff™ Sensory Neuron Maturation Kit (Catalog #100-0684). STEMdiff™ Sensory Neuron Differentiation Kit is used to generate sensory neuron precursors from human pluripotent stem cell (hPSC)-derived neural crest cells (NCCs), which can be obtained using STEMdiff™ Neural Crest Differentiation Kit (Catalog #08610). STEMdiff™ Sensory Neuron Maturation Kit, which contains physiological BrainPhys™ Neuronal Medium as the basal medium, can then be used to mature the sensory neuron precursors into sensory neurons and maintain them for long-term culture.

Cells generated using STEMdiff™ Sensory Neuron System are > 70% positive for class III β -tubulin (neuron marker) and peripherin (peripheral neuron marker). These cells also exhibit activity in response to the sensory ligand capsaicin, indicating a population of functional human PSC-derived sensory neurons that are useful for drug discovery and pain research applications.

Product Information

The following components are sold as part of a complete kit (Catalog #100-0341 or 100-0684) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Sensory Neuron Differentiation Kit (Catalog #100-0341)				
STEMdiff™ Sensory Neuron Differentiation Basal Medium	100-0342	100 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Sensory Neuron Differentiation Supplement	100-0343	1 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Sensory Neuron Maturation Kit (Catalog #100-0684)				
BrainPhys™ Neuronal Medium*	05797	100 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Sensory Neuron Maturation Supplement**	100-0344	25 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

* Protect from light.

** This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
STEMdiff™ Neural Crest Differentiation Kit	08610
ACCUASE™	07920
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg++)	37350
Corning® Matrigel® hESC-Qualified Matrix OR Poly-L-ornithine solution and Laminin	Corning 354277 OR Sigma P4957 and Sigma L2020
STEMdiff™ Neural Crest Differentiation Kit	08610
Trypan Blue	07050
Conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Serological pipettes, 2 mL and 5 mL	e.g. 38002 and 38003

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH POLY-L-ORNITHINE/LAMININ OR CORNING® MATRIGEL®

PLO/Laminin

- Dilute the Poly-L-ornithine (PLO) solution in Dulbecco's phosphate-buffered saline (D-PBS) to reach a final concentration of 15 µg/mL.
For example, add 15 mL of PLO to 85 mL of D-PBS.
- Gently mix diluted PLO solution. Do not vortex.
- Add PLO solution to the tissue culture-treated cultureware to cover the entire growth surface. Refer to Table 1 for recommended coating volumes.
- Gently tilt the cultureware to spread the substrate solution evenly across the surface and incubate at 37°C and 5% CO₂ for 2 hours. Do not let the coating solution evaporate.
NOTE: If not used immediately, cultureware must be sealed to prevent evaporation of the substrate solution (e.g. with Parafilm®). Sealed cultureware can be stored at 2 - 8°C overnight. Allow stored coated cultureware to come to room temperature (15 - 25°C) before proceeding to step 6.
- Prepare a 5 µg/mL working solution of laminin in DMEM/F-12 with 15 mM HEPES. Refer to Table 1 for recommended coating volumes.
- Gently tilt the PLO-coated cultureware onto one side and allow excess PLO solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coating is not scratched.
- Wash the PLO-coated cultureware twice by pipetting D-PBS gently toward the corner of the cultureware to avoid removing the coating.
- Remove the D-PBS from the cultureware and immediately add the laminin solution to cover the entire growth surface.
- Incubate at 37°C and 5% CO₂ for 2 hours. Do not let the laminin solution evaporate.
- NOTE: Using freshly coated cultureware is recommended. However, if not used immediately, sealed cultureware can be stored at 2 - 8°C in laminin solution for up to 4 days after coating. Warm the coated cultureware to 37°C before use.
- Gently remove the laminin solution immediately prior to seeding cells. Do not let the surface dry.
NOTE: It is not necessary to wash cultureware after removing the laminin solution.

Corning® Matrigel®

Corning® Matrigel® should be aliquoted and frozen. Consult the Certificate of Analysis supplied with Corning® Matrigel® for the recommended aliquot size ("Dilution Factor") to prepare 24 mL of diluted matrix. Make sure to always keep Corning® Matrigel® on ice when thawing and handling to prevent it from gelling.

- Thaw one aliquot of Corning® Matrigel® on ice.
- Dispense 24 mL of cold DMEM/F-12 with 15 mM HEPES into a 50 mL conical tube and keep on ice.
- Add thawed Matrigel® to the cold DMEM/F-12 with 15 mM HEPES (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.
- Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. Refer to Table 1 for recommended coating volumes.
- Swirl the cultureware to spread the solution evenly across the surface.
NOTE: If the surface of the cultureware is not fully coated by the Matrigel® solution, it should not be used.
- Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the Matrigel® solution evaporate.
NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the Matrigel® solution (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to come to room temperature before proceeding to step 7.
- Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

Table 1: Recommended Volumes for Coating Cultureware

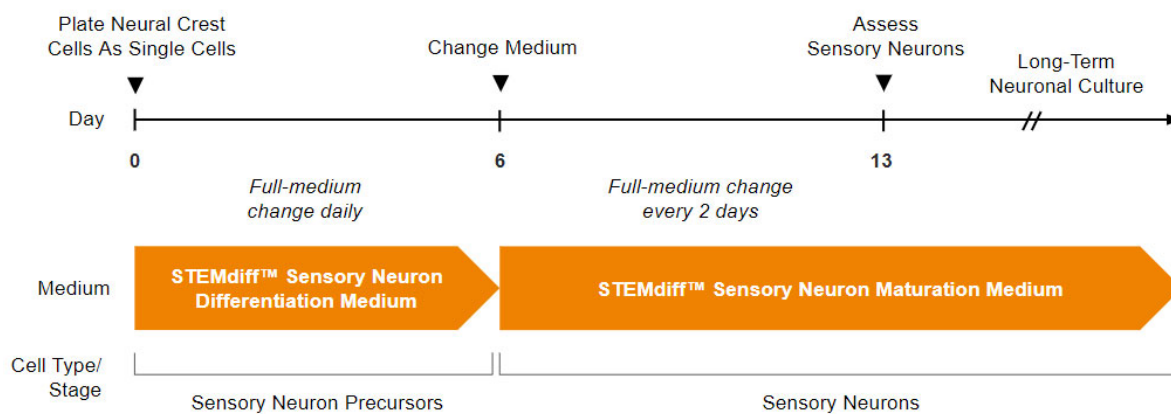
CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF COATING SOLUTION
96-well plate	0.32 cm ² /well	50 µL/well
24-well plate	1.9 cm ² /well	250 µL/well
12-well plate	3.8 cm ² /well	500 µL/well
6-well plate	9.5 cm ² /well	1 mL/well
35 mm dish	9 cm ²	1 mL
60 mm dish	21 cm ²	2.5 mL

B. PREPARATION OF STEMdiff™ SENSORY NEURON DIFFERENTIATION MEDIUM AND MATURATION MEDIUM

Use sterile technique to prepare the following:

- STEMdiff™ Sensory Neuron Differentiation Medium (STEMdiff™ Sensory Neuron Differentiation Basal Medium + STEMdiff™ Sensory Neuron Differentiation Supplement)
 - STEMdiff™ Sensory Neuron Maturation Medium (BrainPhys™ Neuronal Medium + STEMdiff™ Sensory Neuron Maturation Supplement)
1. Thaw STEMdiff™ Sensory Neuron Differentiation Supplement and STEMdiff™ Sensory Neuron Maturation Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.
 NOTE: If not used immediately, store supplements at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the supplements. After thawing aliquots, do not refreeze.
 2. The following example is for preparing 101 mL of STEMdiff™ Sensory Neuron Differentiation Medium. If preparing other volumes, adjust accordingly.
 - a. Add 1 mL of STEMdiff™ Sensory Neuron Differentiation Supplement to 100 mL of STEMdiff™ Sensory Neuron Differentiation Basal Medium. Mix thoroughly.
 - b. Warm medium to room temperature before use.
 NOTE: If not used immediately, store at 2 - 8°C for up to 2 weeks.
 3. The following example is for preparing 125 mL of STEMdiff™ Sensory Neuron Maturation Medium. If preparing other volumes, adjust accordingly.
 - a. Add 25 mL of STEMdiff™ Sensory Neuron Maturation Supplement to 100 mL of BrainPhys™ Neuronal Medium. Mix thoroughly.
 - b. Warm medium to room temperature 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. Use sterile technique when performing the following protocols:

- A. Generation of Neural Crest Cells
- B. Differentiation of Neural Crest Cells to Sensory Neuron Precursors
- C. Maturation of Sensory Neurons

A. GENERATION OF NEURAL CREST CELLS

Refer to the Product Information Sheet for STEMdiff™ Neural Crest Differentiation Kit (Document #10000005429) to generate NCCs, then proceed to section B for differentiation to sensory neurons.

NOTE: Ensure NCCs are ≥ 70% SOX10+ before proceeding to section B. SOX10 expression can be measured by immunocytochemistry (ICC) after labeling with Anti-Sox-10 Antibody (A-2) (Santa Cruz Biotechnology Inc. Catalog #sc-365692).

B. DIFFERENTIATION OF NEURAL CREST CELLS TO SENSORY NEURON PRECURSORS

NCCs are ready for passage after 6 days in STEMdiff™ Neural Crest Differentiation Medium. The following instructions are for generating sensory neurons from a single well of a 6-well plate of neural crest cells. If using other cultureware, adjust volumes accordingly. Cells can be passaged for analysis or downstream differentiation, as follows:

1. Prior to passaging NCCs, coat new cultureware with PLO/laminin or Corning® Matrigel® (Preparation section A).
2. Prepare a sufficient volume of STEMdiff™ Sensory Neuron Differentiation Medium and warm to room temperature. Warm DMEM/F-12 with 15 mM HEPES and ACCUTASE™ to room temperature.
3. Aspirate medium from NCCs and add 1 mL ACCUTASE™ per well.
4. Incubate at 37°C for 5 - 10 minutes.
5. Using a 1 mL pipettor, pipette up and down to dislodge remaining attached cells.
6. Wash wells with 3 - 5 mL DMEM/F-12 with 15 mM HEPES, then transfer the NCC suspension to a 15 mL conical tube.
7. Centrifuge at 300 x *g* for 5 minutes.
8. Carefully remove the supernatant and resuspend the cell pellet in 1 mL of STEMdiff™ Sensory Neuron Differentiation Medium.
9. Perform a viable cell count using Trypan Blue and a hemocytometer.
10. Add the cells to the coated cultureware (prepared in step 1) at 200,000 - 250,000 cells/cm² in STEMdiff™ Sensory Neuron Differentiation Medium.
11. 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 wells.
12. Perform a full-medium change daily with STEMdiff™ Sensory Neuron Differentiation Medium, for 6 days.

B. MATURATION OF SENSORY NEURONS

1. On Day 6, prepare STEMdiff™ Sensory Neuron Maturation Medium, then use it to perform a full-medium change. Incubate at 37°C.
2. Perform a full-medium change every 2 days with STEMdiff™ Sensory Neuron Maturation Medium, for a minimum of 7 days. Cells can be cultured long term (> 30 days) if desired.

Assessment of Sensory Neuron Differentiation

Sensory neuron differentiation efficiency can be optimally assessed between Day 12 - 14 by ICC after labeling with the following antibodies:

- Anti-Beta-Tubulin III Antibody, Clone TUJ1 (Catalog #60052)
- Anti-peripherin antibody, polyclonal (Millipore Catalog #AB1530)

Between Day 12 - 14, > 70% of cells express class III β -tubulin and peripherin. Results may vary depending on cell line used.

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCNCworkflow or contact us at techsupport@stemcell.com.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO WWW.STEMCELL.COM/COMPLIANCE.

Copyright © 2023 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and STEMdiff are trademarks of STEMCELL Technologies Canada Inc. BrainPhys is a registered trademark of the Salk Institute for Biological Studies, used under exclusive license. Corning and Matrigel are registered trademarks of Corning Incorporated. ACCUTASE is a trademark of Innovative Cell Technologies, Inc. Parafilm is a registered trademark of Bemis Company, Inc. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.