

# STEMdiff™ Sensory Neuron Differentiation Kit

## STEMdiff™ Sensory Neuron Maturation Kit



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

## Product Description

STEMdiff™ Sensory Neuron Differentiation Kit (Catalog #100-0341) is used to generate sensory neuron precursors from neural crest cells (NCCs). NCCs can be generated from human pluripotent stem cells (PSCs) using STEMdiff™ Neural Crest Differentiation Kit (Catalog #08610). The sensory neuron precursors are then matured using STEMdiff™ Sensory Neuron Maturation Kit (Catalog #100-0684), which contains physiological BrainPhys™ as the basal medium. The resulting cells are BRN3A+, > 70% TUJ1+, and 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 Catalog #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 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Sensory Neuron Differentiation Supplement	100-0343	1 mL	Store at -20°C.	Stable for 12 months 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 12 months 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 #
ACCUTASE™	07920
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg+++)	37350
Y-27632	72302
Corning® Matrigel® hESC-Qualified Matrix OR Poly-L-Ornithine (PLO) solution and Laminin	Corning 354277 OR Sigma P4957 and Sigma L2020
Trypan Blue	07050

## Preparation of Reagents and Materials

### A. COATING CULTUREWARE WITH CORNING® MATRIGEL® OR POLY-L-ORNITHINE (PLO)/LAMININ PLO/Laminin

1. Dilute PLO solution in phosphate-buffered saline (PBS) to reach a final concentration of 15 µg/mL.
2. Add PLO solution to cultureware to cover the entire growth surface (see Table 1 for required volumes).
3. Distribute the solution evenly and incubate at 37°C and 5% CO<sub>2</sub> for 2 hours or seal the cultureware (e.g. with Parafilm®) and incubate overnight at 2 - 8°C. Do not let the PLO solution evaporate.

4. Prepare a 5 µg/mL working solution of laminin in DMEM/F-12 (see Table 1 for required volumes).
5. Rinse PLO-coated vessel twice with sterile PBS. Pipette PBS gently toward the corner of the cultureware to avoid removing the PLO coating.
6. Aspirate PBS from the cultureware and add the laminin solution to cover the entire growth surface (see Table 1 for required volumes).
7. Incubate at 37°C and 5% CO<sub>2</sub> for 2 hours or seal the cultureware (e.g. with Parafilm®) and incubate overnight at 2 - 8°C. Do not let the laminin solution evaporate.  
 NOTE: Using freshly coated cultureware is recommended. However, if not used immediately, coated cultureware can be stored at 2 - 8°C in laminin solution for up to 4 days.
8. Warm coated cultureware to 37°C before use.
9. Aspirate laminin solution immediately prior to seeding cells. Do not let the surface dry. It is not necessary to wash cultureware after removal of laminin solution.

### Corning® Matrigel®

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

NOTE: Use tissue culture-treated cultureware.

1. Thaw one aliquot of Corning® Matrigel® on ice.
2. Dispense 24 mL of cold DMEM/F-12 into a 50 mL conical tube and keep on ice.
3. Add thawed Matrigel® to the cold DMEM/F-12 (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.
4. Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. See Table 1 for recommended coating volume.
5. 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.
6. 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 for 30 minutes before proceeding to step 7.
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	0.33 cm <sup>2</sup> /well	50 µL/well
4- or 24-well plate	2 cm <sup>2</sup> /well	250 µL/well
6-well plate	10 cm <sup>2</sup> /well	1.5 mL/well
35 mm dish	10 cm <sup>2</sup>	1.5 mL
60 mm dish	20 cm <sup>2</sup>	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™ + 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 100 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™. 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.

## Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols.

### A. GENERATION OF NEURAL CREST CELLS

Refer to the protocol for STEMdiff™ Neural Crest Differentiation Kit (Catalog #08610, Document #10000005429) to generate neural crest cells (NCCs), then proceed to section B for differentiation to sensory neurons.

### B. DIFFERENTIATION OF NEURAL CREST CELLS TO SENSORY NEURONS

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. Coat new cultureware with Corning® Matrigel® or PLO/laminin (Preparation section).
2. Prepare a sufficient volume of STEMdiff™ Sensory Neuron Differentiation Medium and warm to room temperature. Warm DMEM/F-12 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, then transfer the NCC suspension to a 15 mL conical tube.
7. Centrifuge at 300 x g for 5 minutes.
8. Carefully remove and discard the supernatant. Add 1 mL STEMdiff™ Sensory Neuron Differentiation Medium.
9. Count viable cells using Trypan Blue and a hemocytometer.
10. Gently remove the matrix solution from the new coated plate (prepared in step 1) using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
11. Add cells to the new coated plate at 200,000 - 250,000 cells/cm<sup>2</sup> in STEMdiff™ Sensory Neuron Differentiation Medium.
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 wells.
13. Perform a full-medium change daily with STEMdiff™ Sensory Neuron Differentiation Medium, for 5 - 6 days.
14. On day 5/6, prepare STEMdiff™ Sensory Neuron Maturation Medium, then use it to perform a full-medium change. Incubate at 37°C.
15. Perform a full-medium change every 2 - 3 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

For evaluating sensory neuron differentiation, the optimal timepoint for assessment is between days 12 - 14. Antibodies for sensory neuron markers such as anti-peripherin (Millipore AB1530) or anti-Brn3A (Santa Cruz SC-8429) can be used alone or in combination to evaluate the yield of sensory neurons after differentiation. 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](http://www.stemcell.com/hPSCNCworkflow) or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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