# STEMdiff™ Sensory Neuron Differentiation Kit
# STEMdiff™ Sensory Neuron Maturation Kit

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

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## 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 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 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 # |
|---|---|
| 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<br>OR<br>Poly-L-ornithine solution and Laminin | Corning 354277<br>OR<br>Sigma P4957 and Sigma L2020 |
| STEMdiff™ Neural Crest Differentiation Kit | 08610 |
| Trypan Blue | 07050 |

# Preparation of Reagents and Materials

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

### PLO/Laminin

1. Dilute the Poly-L-ornithine (PLO) solution in Dulbecco's phosphate-buffered saline (D-PBS) to reach a final concentration of 15 µg/mL.
2. Gently mix diluted PLO solution. Do not vortex.
3. Add PLO solution to the cultureware to cover the entire growth surface. Refer to Table 1 for recommended coating volumes.
4. Gently tilt the cultureware to spread the substrate solution evenly across the surface and incubate at 37°C and 5% $CO_2$ 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.
5. 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.
6. 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.
7. Wash the PLO-coated cultureware twice by pipetting D-PBS gently toward the corner of the cultureware to avoid removing the PLO coating.
8. Remove the D-PBS from the cultureware and immediately add the laminin solution to cover the entire growth surface.
9. Incubate at 37°C and 5% $CO_2$ 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.
10. Warm the coated cultureware to 37°C before use.
11. 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®

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 with 15 mM HEPES into a 50 mL conical tube and keep on ice.
3. 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.
4. Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. Refer to 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 $cm^2$/well | 50 µL/well |
| 4- or 24-well plate | 2 $cm^2$/well | 250 µL/well |
| 6-well plate | 10 $cm^2$/well | 1.5 mL/well |
| 35 mm dish | 10 $cm^2$ | 1.5 mL |
| 60 mm dish | 20 $cm^2$ | 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)

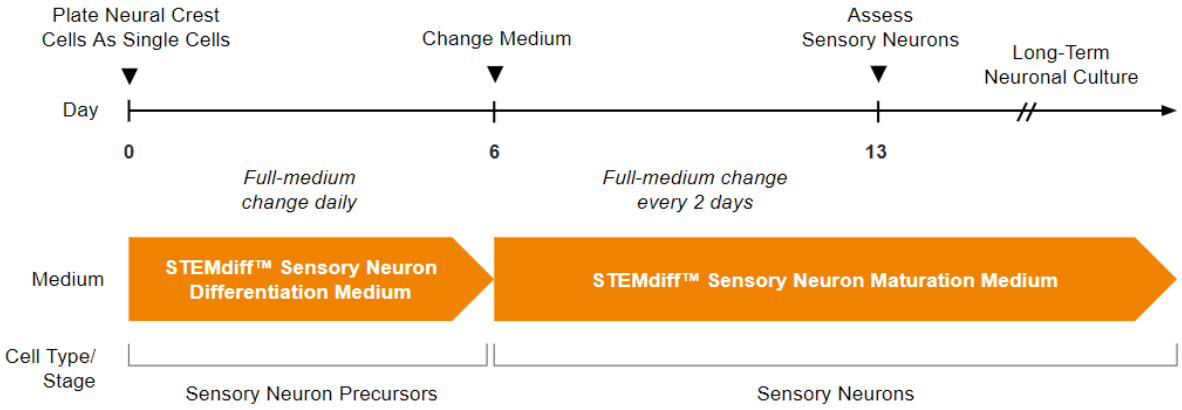
1. Thaw STEMdiff™ Sensory Neuron Differentiation Supplement and STEMdiff™ Sensory Neuron Maturation Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   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 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.

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/cm$^2$ 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.