PRODUCT DESCRIPTION

STEMdiff[™] Neural Progenitor Medium is optimized to support the growth of neural progenitor cells (NPCs). This medium was developed for NPCs derived from human embryonic stem (ES) and induced pluripotent stem (iPS) cells using STEMdiff[™] Neural Induction Medium (Catalog #05831).

COMPONENTS

| 05834 STEMdiff™ Neural Progenitor Basal Medium | 500 mL | |
|--|--------|--|
| 05836 STEMdiff [™] Neural Progenitor Supplement A (50X) | 10 mL | |
| 05837 STEMdiff [™] Neural Progenitor Supplement B (1000X) | 500 µL | |
| These products have been asentically manufactured using tightly | | |

controlled processes and are sterility tested.

STABILITY AND STORAGE

05834 STEMdiff™ Neural Progenitor Basal Medium 500 mL

Product stable at -20°C until expiry date as indicated on label. Once thawed, medium is stable for 3 weeks at 2 - 8°C. Thawed medium can be aliquoted and stored at -20°C. Avoid additional freeze-thaw cycles.

05836 STEMdiff[™] Neural Progenitor Supplement A (50X) 10 mL

Product stable at -20°C until expiry date as indicated on label. Once thawed, supplement can be aliquoted and stored at -20°C. Avoid additional freeze-thaw cycles.

05837 STEMdiff[™] Neural Progenitor Supplement B (1000X) 500 µL

Product stable at -20°C until expiry date as indicated on label. Once thawed, supplement can be aliquoted and stored at -20 C. Avoid additional freeze-thaw cycles.

ADDITIONAL REQUIRED MATERIALS

| PRODUCT | CATALOG # |
|-------------|-----------|
| DMEM/F-12 | 36254 |
| ACCUTASE™ | 07920 |
| Trypan Blue | 07050 |

PREPARATION OF STEMdiff[™] NEURAL PROGENITOR MEDIUM

Use sterile techniques when preparing complete STEMdiff[™] Neural Progenitor Medium. Instructions given in this section are for preparing approximately 100 mL of complete STEMdiff[™] Neural Progenitor Medium.

Add 2 mL of the STEMdiff[™] Neural Progenitor Supplement A and 100 µL of the STEMdiff[™] Neural Progenitor Supplement B to 98 mL of the STEMdiff[™] Neural Progenitor Basal Medium. Mix well.

Note: Complete STEMdiff[™] Neural Progenitor Medium is stable at 2 - 8°C for 2 weeks. Do not freeze complete medium.

This product contains potentially hazardous material. Please refer to the Material Safety Data Sheet (MSDS).



DIRECTIONS FOR USE

Note: Poly-L-ornithine/laminin or BD MatrigelTM-coated plates should be prepared in advance and brought to room temperature (15 - 25°C) for at least 30 min prior to use. For complete instructions on coating plates with these matrices, please refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiffTM Neural System (Document #28782) available on our website at www.stemcell.com or contact us to request a copy.

Note: NPCs are ready for passage when cultures are approximately 80 - 90% confluent.

The following are instructions for passaging cells from one well of a 6-well plate. Indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly.

 Warm (37°C) sufficient volumes of complete STEMdiff[™] Neural Progenitor Medium, DMEM/F-12, and ACCUTASE[™].

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

- 2. Aspirate medium and add 1 mL of ACCUTASE[™].
- 3. Incubate at 37°C for 5 10 minutes.
- 4. Dislodge remaining attached cells by pipetting up and down using a 1 mL micropipette.
- 5. Add 5 mL of DMEM/F-12 and transfer the NPC-suspension to a 15 mL tube.
- 6. Centrifuge at 300 X g for 5 minutes.
- 7. Carefully aspirate the supernatant and add 1 mL of complete STEMdiff[™] Neural Progenitor Medium.
- 8. Count viable cells using Trypan Blue and a hematocytometer.
- Plate cells at desired density (e.g. 1.25 x 10⁵ cells/cm²) in complete STEMdiff[™] Neural Progenitor Medium onto Poly-L-ornithine/laminin or BD Matrigel[™]-coated plates.
- Place the 6-well plate in a 37°C incubator, with 5% CO₂ and 95% humidity. Move the plate in several quick, short, backand-forth and side-to-side motions to evenly distribute the NPCs across the surface of the wells.
- 11. Perform daily medium change using complete STEMdiff[™] Neural Progenitor Medium.
- Visually assess cultures to monitor growth and to determine timing of the next passage (i.e. when cells are approximately 80 - 90% confluent, typically after approximately 7 days of culture).

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