



ENStem-A™ Neural Freezing Medium (1X) For ENStem-A Human Neural Progenitors

CATALOG NUMBER: SCM011

LOT NUMBER:

QUANTITY: 50 mL

DESCRIPTION: ENStem-A™ Neural Freezing Medium is qualified for use with ENStem-A™ Human Neural Progenitor Cells (Part No. SCC003) cultured in defined serum-free conditions with ENStem-A™ Neural Expansion Medium (Part No. SCM004). The optimized formulation allows for consistent cryopreservation and high viability upon thawing and plating.

APPLICATIONS: Cryopreservation of ENStem-A Human Neural Progenitor Cells in defined serum-free conditions.

PROTOCOL:

1. Thaw cell culture freezing medium completely and mix well by gently swirling the bottle. Keep freezing medium on ice during use.
2. Cells to be frozen should be in late log phase growth.
3. Monolayers will need to be dissociated with Accutase (Part No. SCR005). After dissociation, cells are resuspended in ENStem-A Neural Expansion Medium (Cat. No. SCM004) and counted to determine viability and number.
4. Centrifuge cells at 300 xg for 3 min. Remove the medium above the pellet.
5. Resuspend the cells in ENStem-A Neural Freezing Medium at a concentration of $\sim 3 \times 10^6$ cells/mL. Freeze 1 mL of cells/vial. After the cells have been resuspended and aliquoted into appropriate cryogenic storage vials, they can be placed in a freezing container and the normal freeze down procedure should be started within five minutes.
6. Cells must be stored at or below -80°C . For long term storage the cells should be stored in ultra-low temperature freezer (-150°C), or in liquid nitrogen (-196°C).
7. Thawing of cryopreserved cells should be as follows:
 - a. Do not thaw the cells until the recommended medium and appropriately coated poly-L-ornithine and laminin plasticware and/or glassware are on hand.
 - b. Thaw cells quickly in a 37°C water bath. **Important: Do not vortex the cells.**
 - c. Sterilize vial by rinsing with 70% ethanol.
 - d. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.
 - e. Using a 10 mL pipette, slowly add dropwise 9 mL of ENStem-A Neural Expansion Medium (Part No. SCM004) (pre-warmed at 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of medium at once to the cells. This may result in decreased cell viability due to osmotic shock.**
 - f. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT: Do not vortex the cells.**
 - g. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
 - h. Decant as much of the supernatant as possible



- i. Resuspend the cells in a total volume of 2 mL of ENStem-A Neural Expansion Medium (Part No. SCM004) (pre-warmed at 37°C) containing freshly added L-Glutamine (2 mM) and FGF-2 (20 ng/mL).

Note: *Growth factors should always be added fresh to ENStem-A Neural Expansion Medium.*

- j. Plate the cell mixture onto a poly-L-ornithine and laminin-coated 3.5-cm tissue culture plate. **IMPORTANT: For optimal growth, ENStem-A cells should be maintained at high cell density at all times. Thus we do not recommend thawing the cells on tissue culture plates that are larger than a 3.5-cm tissue culture plate.**
- k. Incubate the cells at 37°C in a 5% CO₂ humidified incubator.
- l. The next day, exchange the medium with fresh ENStem-A Neural Expansion Medium (pre-warmed to 37°C) containing L-Glutamine (2 mM) and FGF-2 (20 ng/mL). Exchange with fresh medium containing L-Glutamine and FGF-2 every other day thereafter.
- m. When the cells are approximately 90-100% confluent, they can be dissociated manually or enzymatically with Accutase™ (Part No. SCR005) and passaged or alternatively frozen for later use. The cells should be maintained at high cell density at all times and thus the recommended passaging is at 1:2.

PRESENTATION: Serum-free formulation. Contains 10% DMSO.

**STORAGE/
HANDLING:** Store at -20°C. Refer to lot expiration date on label.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION**

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