**INTENDED USE**

PRIME-XV™ NPC Expansion XSFM is intended for use in the maintenance and expansion of human neural progenitor cells (NPCs) under serum-free and xeno-free culture conditions while maintaining their ability to differentiate into neural, oligodendrocyte and astrocyte lineages. This medium is ready to use and can be supplemented with additional cytokine/growth factors when desired for specific applications.

**PRODUCT DESCRIPTION**

PRIME-XV™ NPC Expansion XSFM is a serum-free, xeno-free medium optimized for maintenance and expansion of human neural progenitor cells. This product does not contain antibiotics.

**SHIPPING**

This product is shipped with dry ice. Upon receipt, store it immediately at the temperature recommended below.

**STORAGE INSTRUCTIONS AND STABILITY**

Upon receipt, store the medium at or below - 10 °C in a manual defrost freezer. Unopened medium is stable for 6 months from date of receipt when stored at or below - 10 °C in a manual defrost freezer. PRIME-XV™ NPC Expansion XSFM can be aliquoted and stored at or below - 10 °C in a manual defrost freezer for up to 3 months. When ready to use, thaw this medium overnight at 2 - 8 °C in the dark. PRIME-XV™ NPC Expansion XSFM should be used within one week when stored at 2 - 8 °C and protected from light. Repeated freeze-thaw cycles should be avoided.

**PRECAUTIONS AND WARNINGS**

This product is for research or further manufacturing use only. It is not for use in diagnostic procedures. The safety and efficacy of this product in diagnostic or other clinical uses has not been established. This reagent should not be used beyond the expiration date. Results may vary due to variations among human NPCs derived from different donors.

This product contains transferrin derived from human plasma, which has been tested and found negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). However, the medium should be handled as if potentially infectious. Safe laboratory procedures should be followed and protective clothing should be worn when handling this medium. The acute and chronic effects of over-exposure to this medium are unknown.

**DIRECTIONS FOR USE**

These procedures are general guidelines for culturing human NPCs isolated from fetal tissue or embryonic stem cells as well as for murine NPCs isolated from fetuses or post-natal brains. Procedures for optimal growth conditions should be determined for each application and cell type as appropriate.

Neural progenitor cells may be grown in PRIME-XV™ NPC Expansion XSFM (Irvine Scientific, Catalog #91131) in monolayer culture on precoated tissue culture treated vessels or as neurospheres on untreated low adhesion tissue culture vessels.

- **Coating Procedures**
  
  Note: To grow neural progenitor cells in monolayer, culture vessels have to be pre-coated with substrate for cell attachment. It is highly recommended to use Poly-L-Omithine and PRIME-XV™ Human Fibronectin (Irvine Scientific, Catalog #3102) to coat culture surfaces for consistency. Other types of matrix substrate (PRIME-XV™ MatrisF, Irvine Scientific, Catalog #31001) may be used. Types and amounts of matrix proteins are dependent on the experimental design of each individual investigator.

  1. Dissolve Poly-L-omithine in sterile PBS (Irvine Scientific, Catalog #9236) to make a 15 mg/mL stock (1000X). Aliquot and store at ≤-20 °C.
  2. Dilute the 1000X Poly-L-omithine stock 1000-fold in sterile PBS (Irvine Scientific, Catalog #9236) to make a 15 µg/mL (1X) solution. Prepare fresh as needed.
  3. Add the (1X) Poly-L-omithine solution to culture vessel at a ratio of 0.15 mL/cm². Incubate 3 hours to overnight at 37°C and 5% CO₂.
  4. Discard the Poly-L-omithine solution. Wash each vessel 3 times with equal amount of PBS (Irvine Scientific, Catalog #9236).
  5. Add PBS to each vessel at a ratio of 0.15 mL/cm². Incubate overnight at 37°C and 5% CO₂.
  6. Allow the vial of Human Fibronectin (Irvine Scientific, Catalog #31002) to warm to room temperature without agitation. Make a 10 µg/mL solution pipetting the human fibronectin into sterile PBS and gently inverting the tubes. Prepare fresh as needed.
  7. Discard the PBS from each Poly-L-omithine coated dish. Wash once with PBS.
  8. Add the 10µg/mL human fibronectin solution to each dish at a ratio of 0.15 mL/cm². Incubate at 37°C and 5% CO₂ for 3 hours to overnight.

- **Expanding and Sub-culturing Human NPC in PRIME-XV™ NPC Expansion XSFM**

  **Monolayer adherent cultures**

  1. Add thawed cell suspension to a pre-coated culture vessel (see coating procedure) at a seeding density of 2-5 x 10⁶ viable cells/cm² in appropriate volume of medium for the culture vessel.
  2. Incubate the cells in a 37°C, 5% CO₂ incubator.
  3. 24 hours post-thaw, remove the spent medium from the plate and add pre-warmed PRIME-XV™ NPC Expansion XSFM (3-5 drops per second) while gently swirling.
  4. Pipette the content of the entire vial into a 15 ml conical tube. Add 5 to 10 ml pre-warmed PRIME-XV™ NPC Expansion XSFM (3-5 drops per second) while gently swirling.
  5. Centrifuge the cells at 200xg for 10 minutes at room temperature.
  6. Re-suspend the pellet in a minimum volume of pre-warmed PRIME-XV™ NPC Expansion XSFM, count the cells and determine total viable cell number.

- **Recovery of Cryopreserved Human NPCs**

  1. Thaw PRIME-XV™ NPC Expansion XSFM at room temperature. Pre-warm at 37°C the amount of medium needed for one procedure (repeated warming of medium may reduce product performance). Store the remaining medium at 2-8°C. Thawed medium can be kept in the refrigerator for up to 7 days.
  2. Pre-coat the tissue culture vessel following the protocol above. Note: for neurosphere suspension cultures, use low or ultra-low attachment surface culture vessels.
  3. Rapidly thaw a frozen vial of NPCs in a 37°C water bath while swirling the vial until all its content is liquid. The vial should still feel cold to touch. The process typically takes less than 2 minutes.
  4. Pipette the content of the entire vial into a 15 ml conical tube. Add 5 to 10 ml pre-warmed PRIME-XV™ NPC Expansion XSFM drop-wise (3-5 drops per second) while gently swirling.
  5. Centrifuge the cells at 200xg for 10 minutes at room temperature.
  6. Re-suspend the pellet in a minimum volume of pre-warmed PRIME-XV™ NPC Expansion XSFM, count the cells and determine total viable cell number.

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  3. Rapidly thaw a frozen vial of NPCs in a 37°C water bath while swirling the vial until all its content is liquid. The vial should still feel cold to touch. The process typically takes less than 2 minutes.
  4. Pipette the content of the entire vial into a 15 ml conical tube. Add 5 to 10 ml pre-warmed PRIME-XV™ NPC Expansion XSFM (3-5 drops per second) while gently swirling.
  5. Centrifuge the cells at 200xg for 10 minutes at room temperature.
  6. Re-suspend the pellet in a minimum volume of pre-warmed PRIME-XV™ NPC Expansion XSFM, count the cells and determine total viable cell number.

- **Expanding and Sub-culturing Human NPC in PRIME-XV™ NPC Expansion XSFM**

  **Monolayer adherent cultures**

  1. Add thawed cell suspension to a pre-coated culture vessel (see coating procedure) at a seeding density of 2-5 x 10⁶ viable cells/cm² in appropriate volume of medium for the culture vessel.
  2. Incubate the cells in a 37°C, 5% CO₂ incubator.
  3. 24 hours post-thaw, remove the spent medium from the plate and add pre-warmed PRIME-XV™ NPC Expansion XSFM.
  4. Change the medium in the culture vessel with pre-warmed medium every two days.
  5. Subculture when the cells reach 80-90% confluency.
  6. Pre-warm PRIME-XV™ NPC Expansion XSFM and a cell dissociation reagent (i.e. Accutase*) at 37°C.
  7. Aspirate the medium from the culture flask.

Refer to the following table for specific culture vessels:
8. Rinse the cells with sterile PBS without calcium and magnesium (Irvine Scientific, Catalog #9240).

9. Add ~ 0.1 mL/cm$^2$ of pre-warmed Accutase* to the flask making sure the solution covers the entire culture surface. Incubate at 37°C for 2-5 minutes; check the flask after 2 minutes, the cell layer should start to detach from the culture vessel surface.

10. Once the cells are detached, very gently pipette them up and down to create a single cell suspension. Minimize cells exposure to air by avoiding air bubbles.

11. Add 10 mL of pre-warmed PRIME-XV™ NPC Expansion XSFM and mix gently; transfer the cell suspension to a 15 mL conical tube.

12. Centrifuge the cell suspension at 200xg for 10 minutes.

13. Aspirate the medium and gently resuspend the cell pellet in minimum volume of pre-warmed PRIME-XV™ NPC Expansion XSFM. Count the cells to determine the number of total viable cells.

14. Seed the cells in a pre-coated culture vessel at a density of 2-5x10$^4$ viable cells/cm$^2$ and incubate in a 37°C and 5% CO$_2$ humidified incubator.

15. Remove and discard spent medium and re-feed the cells with pre-warmed medium every 2 days.

Neurosphere suspension cultures

1. Add thawed cell suspension (from Recovery of Cryopreserved Human NPCs section) to a low or ultra low attachment surface culture vessel at a seeding density of >2 x 10$^5$ viable cells/cm$^2$.

   Note: the initial seeding density will impact the number of neurospheres. Low cell viability (less than 90%) will result in increased number of dead cells and debris in the culture suspension.

2. Without removing neurospheres, gently feed the neurosphere culture every 2 - 3 days with pre-warmed PRIME-XV™ NPC Expansion XSFM. The cells within the neurospheres should look bright and almost transparent under the microscope. Neurospheres should be harvested after 3 - 4 days of culture for easy dissociation.

3. When the neurospheres reach a size of 100-200 µm, aspirate suspension culture and transfer to a 15 ml conical tube. Let the neurospheres settle down by gravity and carefully aspirate most of the culture medium. Alternatively, centrifuge at 100xg for 2 minutes and remove supernatant without exposing neurospheres to air.

4. Add 5 mL of sterile PBS without calcium and magnesium and let the neurosphere settle down or centrifuge as in 3 and remove supernatant without exposing neurospheres to air.

5. Add 1 mL of pre-warmed Accutase* and incubate for 15 minutes in a water bath at 37°C.

6. After incubation, pipette neurospheres up and down 10 to 20 times to create a single cell suspension. Add 5-10 mL of PRIME-XV™ NPC Expansion XSFM.

7. Remove a sample to count cells to determine cell density and viability, and centrifuge at 200xg for 10 minutes.

8. Resuspend the cells in pre-warmed PRIME-XV™ NPC Expansion XSFM at the desired density and add to culture vessel. Incubate in a 37°C and 5% CO$_2$ humidified incubator.

*Accutase is a registered trade mark of ICT Inc.

DATA

Nestin-expressing human neural progenitor cells after culture in PRIME-XV™ NPC Expansion XSFM. The cells were cultured on PRIME-XV™ Human Fibronectin coated culture vessel. Nuclei were counterstained with DAPI. (Courtesy of Dr. Shuxian Hu, Infectious Diseases and International Medicine, Medical School, University of Minnesota).