PRIME-XV NPC Expansion XSFM

Catalog #	Product	Size
91131	PRIME-XV NPC Expansion XSFM	250 mL liquid

Intended Use

For research or further manufacturing use only. Not for injection or diagnostic procedures.

Product Description

PRIME-XV NPC Expansion XSFM is a xeno-and–serum-free medium optimized for the cultivation and expansion of neural progenitor cells (NPC) that are able to maintain their ability to differentiate into neural lineages. This medium is ready to use and can be supplemented with additional cytokine/growth factors according to desired applications and does not contain antibiotics.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis which is available at www.irvinesci.com or upon request.

Shipping

This product is shipped with dry ice. Upon receipt, store immediately at or below -10°C in a manual defrost freezer and protect from light.

Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. Unopened PRIME-XV NPC Expansion XSFM is stable for 24 months from date of manufacture, as indicated on the label, when stored at or below -10°C in a manual defrost freezer and protected from light. 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. Repeated freeze thaw cycles should be avoided. When ready to use, thaw overnight at 2-8°C. PRIME-XV NPC Expansion XSFM should be used within one week when stored at 2-8°C and protected from light. This product has not been validated for use beyond the unopened expiry shelf life. Further supplementation with additional cytokine/growth factors can be performed for desired applications.

Precautions

The safety and efficacy of this product has not been established for use in diagnostic or other clinical applications. Please refer to the Safety Data Sheet for information regarding hazards and safe handling practices. PRIME-XV NPC Expansion XSFM should be handled as biohazardous and potentially infectious. Safe laboratory practices should be observed and appropriate personal protective equipment should be worn when handling this medium. The acute and chronic effects of over-exposure to this product are unknown. 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). This medium should not be used beyond the expiration date. Results may vary due to variations among NPCs derived from different donors.

Directions for Use

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

Culture Vessel Coating Procedure

For monolayer culture of rat cortical NPC, culture vessels have to be pre-coated with a substrate for cell attachment. It is recommended that Poly-L-Ornithine and PRIME-XV Human Fibronectin (IS, Catalog #31002) be used as the matrix substrate for coating consistency. Other types of matrix substrate may be used. Types and amounts of matrix proteins are dependent on the experimental design of each individual investigator.

1. Dissolve Poly-L-Ornithine in sterile PBS (IS, Catalog #9236) to make a 15 mg/mL stock (1000X). Aliquot and store at ≤ -20˚C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

2. Dilute the 1000X Poly-L-Ornithine stock 1000-fold in sterile PBS to make a 15 µg/mL 1X solution. Prepare fresh as needed.

3. Add the 1X Poly-L-Ornithine solution to the culture vessel at a ratio of 0.15 mL/cm^2. Incubate for 3 hours to overnight at 37˚C and 5% CO_2.

4. Discard the Poly-L-Ornithine solution. Wash each vessel 3 times with equal amounts of PBS.

5. Add PBS to each vessel at a ratio of 0.15 mL/cm^2. Incubate overnight at 37˚C and 5% CO_2.

6. Allow the vial of PRIME-XV Human Fibronectin to warm to room temperature without agitation. Make a 10 µg/mL solution by adding the fibronectin into sterile PBS and gently pipetting to mix. Prepare fresh as needed.

7. Discard the PBS from each Poly-L-ornithine coated dish. Wash once with PBS.

8. Add the 10 µg/mL fibronectin solution to each dish at a ratio of 0.15 mL/cm^2. Incubate at 37˚C and 5% CO_2 for 3 hours to overnight. Refer to the following table for respective volumes according to specific culture vessels:

Culture Vessel	Surface Area (cm^2)	Volume of 10 µg/mL Human Fibronectin
6-well plate	9.6	1.44 mL/well
T-25 flask	25	3.75 mL
T-75 flask	75	11.25 mL

9. Discard the human fibronectin solution. Wash each vessel with PBS once before use.

NOTE: The PBS washed vessel may be used immediately or stored at 4°C (in PBS), wrapped tightly with Parafilm® for up to 3 weeks.

Recovery of Cryopreserved Cells

The following is a guideline based on the cryopreservation of rat cortical NPC. Specific procedures provided by the respective cell supplier may also be utilized according to tissue source and user preference and need.

1. Prepare complete medium as described above and pre-warm 20 mL in a 37°C water bath.
2. Add 9 mL of pre-warmed PRIME-XV NPC Expansion XSFM to a 15 mL tube.
3. Carefully remove the frozen vial of rat cortical NPCs from liquid nitrogen. Immediately pipette 1 mL of fresh pre-warmed media aseptically to the vial and gently pipet up and down. As the cells begin to thaw, transfer the thawed portion into the remaining pre-warmed media in the 15 mL tube. Repeat this process with the warmed media until all of the cells have thawed.

   NOTE: Rapid resuspension of frozen cells in pre-warmed media during thaw is critical. Allowing cells to thaw in DMSO will dramatically reduce viability.
4. Mix 10 µL of the cell suspension with 10 µL of Trypan Blue and count the live cells on a hemocytometer.
5. Seed the cells in the appropriate culture vessel and cell density based on desired application. For monolayer culture versus neurosphere suspension culture guidelines, see below under section 'Expansion and Sub-culturing.'

Adherent Monolayer Culture

The following is a guideline based on the monolayer culture of rat cortical NPC. Specific procedures provided by the respective cell supplier may also be utilized according to tissue source and user preference and need.

1. Seed the cells onto a pre-coated culture vessel (see Coating Procedure) at a seeding density of 1.7-2.5x10^4 cells/cm^2 (or according to suggested density recommendations from the supplier).

   NOTE: Do not centrifuge cells. Frozen cells are extremely sensitive upon recovery from cryopreservation.
2. Incubate the cells in a 37°C, 5% CO_2 humidified incubator.
3. After the cells have become adherent (3 hours to overnight), replace with fresh pre-warmed complete medium, then subsequently change medium every 2-3 days.
4. Observe the cells regularly to assess survival and viability.
5. Passage the cells once a confluency of 70-80% is reached. Do not allow the cultures to become over confluent. Subculturing under suboptimal conditions may affect product performance.
6. Pre-warm appropriate amount of PRIME-XV NPC Expansion XSFM in a 37°C water bath.
7. Pre-warm cell dissociation reagent (e.g. HBSS without calcium and magnesium, IS, Catalog #9228) at 37°C.

   NOTE: Other dissociation reagents can be used according to user requirements and applications.
8. Aspirate medium from the culture vessel. Rinse the cells with sterile PBS (PBS without calcium and magnesium, IS, Catalog #9240).
9. Add 0.1 mL/cm^2 of pre-warmed HBSS without calcium and magnesium. Make sure the solution covers the entire culture surface area. Incubate at 37°C for 10 minutes.
10. Once the cells are detached, tap the flask and very gently pipette them up and down to create a single cell suspension.

11. Transfer the single cell suspension to an appropriate centrifuge tube.

12. Rinse culture vessel with equal volume of pre-warmed complete medium and add to the centrifuge tube.

13. Centrifuge the cell suspension at 200xg for 5 minutes.

14. Aspirate the supernatant and gently resuspend the cell pellet in 5 mL of pre-warmed complete medium.

15. Perform cell count.

16. Resuspend into pre-warmed complete medium and seed a pre-coated culture vessel (see Coating Procedures) at a density of 1.7-2.5x10^4 cells/cm^2 (or according to suggested density recommendations from supplier).

17. Incubate the cells in a 37°C, 5% CO_2 humidified incubator.

18. Replace with fresh, pre-warmed PRIME-XV NPC Expansion XSFM every 2-3 days.

Neurosphere Suspension Culture

The following is a guideline based on the neurosphere suspension culture of rat cortical NPC. Specific procedures provided by the respective cell supplier may also be utilized according to tissue source and user preference and need.

1. Seed the cells in a low or ultra-low attachment surface culture vessel at a density of >2x10^5 cells/cm^2.

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

2. Taking care not to remove any neurospheres, gently feed the neurosphere culture every 2-3 days with pre-warmed PRIME-XV NPC Expansion XSFM according to the number of neurospheres present (see table below). The cells within the neurospheres should look bright and slightly transparent under the microscope. Neurospheres should be harvested after 4-7 days of culture for easy dissociation.

Number of Neurospheres	Type of Media Exchange
< 50	50%, 1:1 ratio of fresh media to spent media
> 50	100% complete media exchange

3. When the neurospheres reach a size of 100-200 µm or have a dark clump inside and/or ruffling on the outside, aspirate suspension culture and transfer to a 15 mL conical tube. Let the neurospheres settle down by gravity and carefully aspirate 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 (IS, Catalog #9240) and allow the neurospheres to settle down or centrifuge at 100xg for 2 min and remove supernatant without exposing neurospheres to air.

5. Add 1 mL of pre-warmed HBSS without calcium and magnesium (IS, Catalog #9228) and incubate for 15 minutes at 37°C.

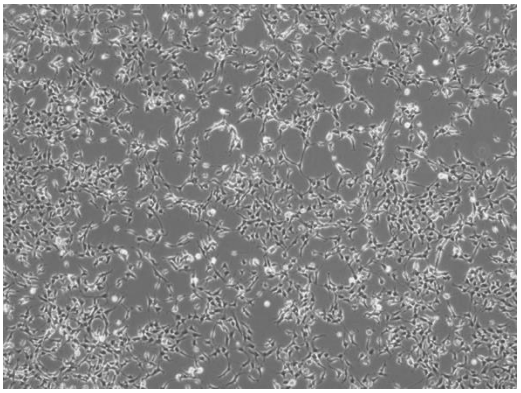
   NOTE: Other dissociation reagents can be used according to user requirements and applications.

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.

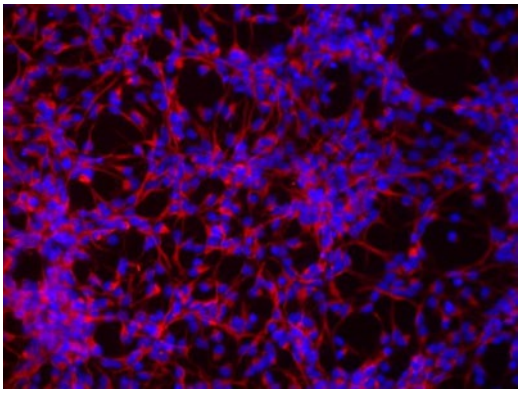
7. Remove a sample to count cells to determine cell density and viability, and centrifuge at 200xg for 10 minutes.

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.

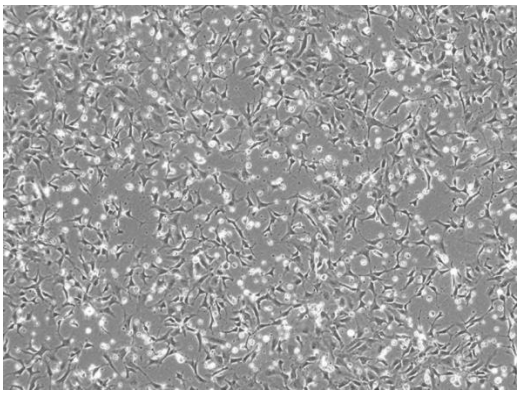
Sample Data



PRIME-XV NPC Expansion XSFM maintains characteristic morphology of rat cortical neural progenitor cells. Rat cortical NPC were cultured in PRIME-XV NPC Expansion XSFM. The cells were plated at a density of $2x10^4$ cells/cm^2. Images were taken on day 5 of culture at 10X magnification.



PRIME-XV NPC Expansion XSFM maintains Nestin expression in rat cortical neural progenitor cells. Rat cortical NPC were cultured in PRIME-XV NPC Expansion XSFM at a density of $7.9x10^4$ cells/cm^2 and cultured for 5 days. The cells were labelled with goat anti-Nestin (R&D Systems, Catalog# AF2736) to detect NPC (red). The nuclei were counterstained with DAPI (blue). Immunofluorescence images were taken at 10X magnification.



PRIME-XV NPC Expansion XSFM maintains characteristic morphology of human neural progenitor cells. Human iPSC-derived neural stem cells were cultured in PRIME-XV NPC Expansion XSFM. The cells were plated at a density of $5x10^4$ cells/cm^2. Images were taken on day 2 of culture at 10X magnification.

Related Products

Catalog #	Product	Size
91142	IS21 Supplement (50X)	10 mL liquid
91201	PRIME-XV Neural Basal Medium	500 mL liquid
9317	L-Glutamine Solution (200 mM)	100 mL, 500 mL liquid
9228	HBSS without calcium and magnesium	100 mL, 500 mL liquid
9240	PBS without calcium and magnesium	500 mL, 1L liquid
9236	PBS with calcium and magnesium	500 mL, 1L liquid

Technical Support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: fisitmrequest@fujifilm.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificate of Analysis (CoA) (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

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