PRIME-XV Neural Basal Medium

Catalog #	Product	Size
91201	PRIME-XV Neural Basal Medium	500 mL liquid

Intended Use

For research or further manufacturing use only.

Product Description

PRIME-XV Neural Basal Medium is a chemically-defined basal medium optimized for the culture and maintenance of neuronal cells when supplemented with PRIME-XV IS21 Supplement (50X). This medium does not contain antibiotics or L-glutamine and can be further supplemented (e.g. N-2 Supplement, growth factors, etc.) according to desired applications, such as neural stem/progenitor cell culture and expansion.

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 at 2-8°C. Upon receipt, store immediately at 2-8°C and protect from light.

Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. Unopened liquid medium is stable for 24 months from date of manufacture, as indicated on the label, when stored at 2-8°C and protected from light. PRIME-XV Neural Basal Medium requires further supplementation with L-glutamine, a serum-free supplement such as PRIME-XV IS21 Supplement (50X), and/or additional cytokine/growth factors according to the desired application(s). Once supplemented, the complete medium should be used within two weeks when stored at 2-8°C and protected from light. This product has not been validated for use beyond the unopened expiry shelf life.

Precautions

The safety and efficacy of this product in diagnostic or other clinical uses has not been established. Please refer to the Safety Data Sheet for information regarding hazards and safe handling practices. PRIME-XV Neural Basal Medium 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.

Directions for Use

Neural Stem/Progenitor Cell Culture and Expansion (Monolayer Culture)

The following monolayer culture protocol has been utilized for the expansion of rat neural progenitor cells (NPC) derived from fetal neural cortical tissue but can be adapted for NPC derived from other sources. Further optimization may be required depending on the cell type and application.

Culture Vessel Coating Procedure

For monolayer culture of rat cortical NPC, culture vessels have to be pre-coated with substrate for cell attachment. It is recommended to use Poly-L-Ornithine and PRIME-XV Human Fibronectin (PN# 31002) as the matrix substrate and 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 (PN# 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.

Complete Medium Preparation

For expansion of rat cortical NPC, prepare complete medium by supplementing the PRIME-XV Neural Basal Medium (PN# 91201) with 2 mM L-Glutamine Solution (PN# 9317) and a serum-free supplement such as N-2 Supplement (100X) (Thermo Fisher Scientific, Catalog# 17502) along with recombinant EGF and/or FGF-basic. To prepare 100 mL of complete medium:

1. Aseptically add 1.0 mL of N-2 Supplement (100X) to 99 mL of PRIME-XV Neural Basal Medium.
2. Aseptically add 0.25 mL of 200 mM L-Glutamine Solution to a final concentration of 0.5 mM to the medium before use.
3. Aseptically add recombinant EGF and/or FGF-basic to a final concentration of 20 ng/mL for each.

   NOTE: Add fresh each time before use.
4. Store the complete medium in the dark at 2-8°C for up to two weeks.

   NOTE: Add component amounts proportionally according to desired volume.

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 10 mL of pre-warmed complete medium to a 15 mL tube.
3. Carefully remove the frozen vial of primary neurons 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 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).

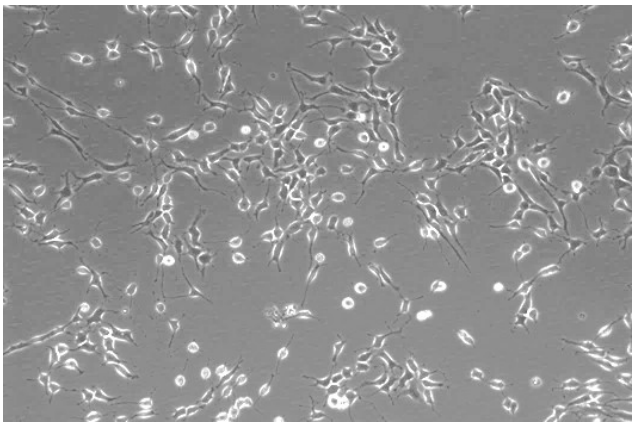
   NOTE: Do not centrifuge cells. Frozen cells are extremely sensitive upon recovery from cryopreservation.
6. Incubate the cells in a 37°C, 5% CO_2 humidified incubator.
7. After the cells have become adherent (3 hours to overnight), replace with fresh pre-warmed complete medium, then subsequently change medium every 2-3 days.
8. Observe the cells regularly to assess survival and viability.
9. 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.

Passaging Cells

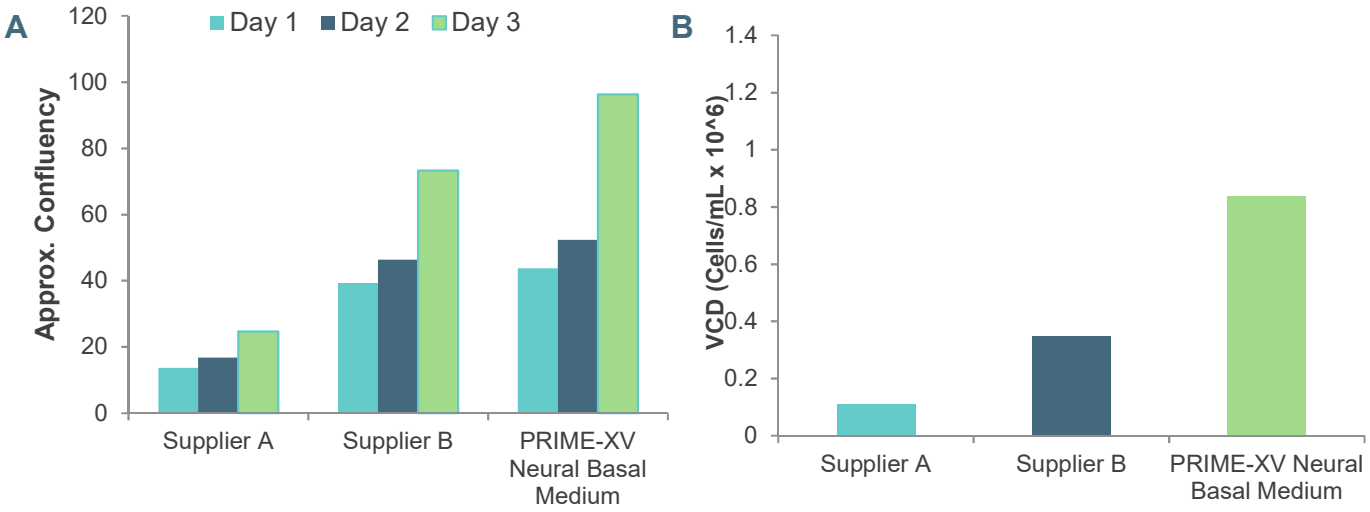
1. Pre-warm 20 mL of complete medium (see Complete Medium Preparation) in a 37°C water bath.
2. Pre-warm cell dissociation reagent (e.g. HBSS without calcium and magnesium, PN# 9228) at 37°C.

   NOTE: Other dissociation reagents can be used according to user requirements and applications.
3. Aspirate medium from the culture vessel. Rinse the cells with sterile PBS (PBS without calcium and magnesium, PN# 9240).
4. 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.
5. Once the cells are detached, tap the flask and very gently pipette them up and down to create a single cell suspension.
6. Transfer the single cell suspension to an appropriate centrifuge tube.
7. Rinse culture vessel with equal volume of pre-warmed complete medium and add to the centrifuge tube.
8. Centrifuge the cell suspension at 200 x g for 10 minutes.
9. Aspirate the supernatant and gently resuspend the cell pellet in 5 mL of pre-warmed complete medium.
10. Perform cell count.
11. 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).
12. Incubate the cells in a 37°C, 5% CO_2 humidified incubator.
13. Replace with fresh, pre-warmed complete medium every 2-3 days.

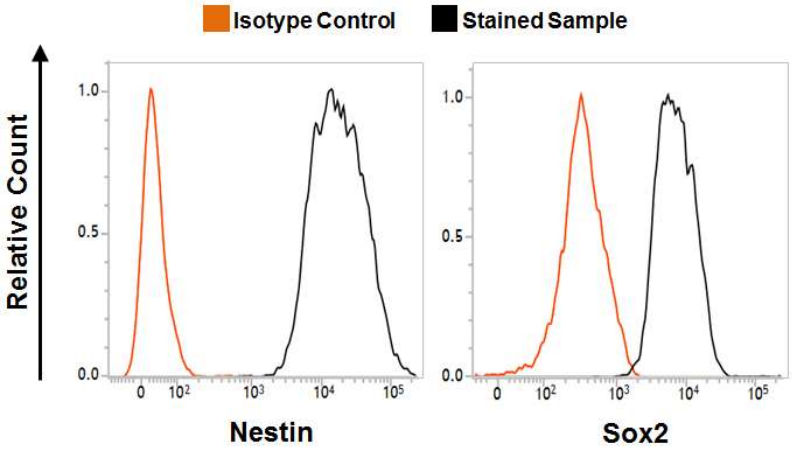
Sample Data



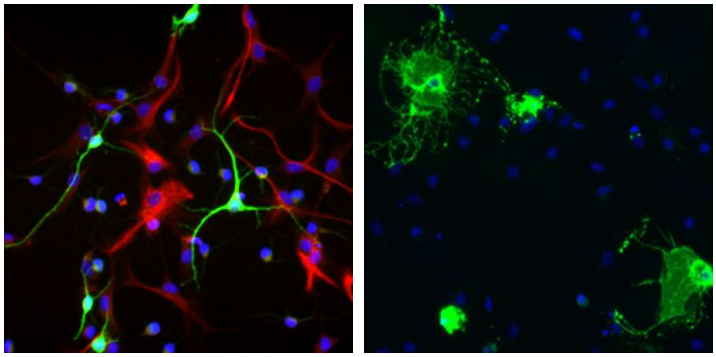
PRIME-XV Neural Basal Medium maintains characteristic morphology of rat cortical neural progenitor cells.
Rat cortical NPC were cultured in PRIME-XV Neural Basal Medium supplemented with L-glutamine, N-2 Supplement, and recombinant FGF-basic. The cells were plated at a density of 20,000 cells/cm^2. Images were taken on day 4 of culture at 20X magnification.



PRIME-XV Neural Basal Medium supports rat cortical neural progenitor cell growth.
Rat cortical NPC were cultured in PRIME-XV Neural Basal Medium as well as two leading supplier's serum-free basal medium supplemented with L-glutamine, N-2 Supplement, recombinant EGF, and recombinant FGF-basic. Approximate confluency was determined for 3 consecutive days using a CSI imager (Molecular Devices) **(A)**. On day 3 of culture, cells were dissociated and processed for viable cell count **(B)**.



PRIME-XV Neural Basal Medium maintains neural progenitor cell marker expression profile over multiple passages.
Rat cortical NPC were cultured in PRIME-XV Neural Basal Medium supplemented with L-glutamine, N-2 Supplement, recombinant EGF, and recombinant FGF-basic. Marker expression profile was assessed by flow cytometry. The cells were stained with mouse anti-mouse/rat Nestin PE conjugated antibody (R&D Systems, Clone 307501) and mouse anti-human/mouse SOX2 monoclonal antibody (R&D systems, Clone 245610) followed by APC conjugated anti-mouse IgG secondary antibody. NSC markers (positive for Nestin and Sox2) were retained throughout multiple passages (P3).



PRIME-XV Neural Basal Medium retains multi-lineage differentiation potential of rat cortical neural progenitor cells. Rat cortical NPC were cultured in PRIME-XV Neural Basal Medium supplemented with N-2 Supplement but no mitogens to induce differentiation. The differentiated cells were labeled with **(A)** sheep anti-human/rat GFAP (R&D Systems, Catalog #AF2594) to detect astrocytes (red), and mouse neuron-specific anti-beta III Tubulin (R&D Systems, Clone Tuj1) to detect neurons (green) and **(B)** mouse anti-oligodendrocyte marker O4 (R&D Systems, Clone O4) to detect oligodendrocytes (green). The nuclei were counterstained with DAPI (blue). Immunofluorescence images were taken at 20X magnification.

Neuronal Cell Maintenance and Differentiation

The following protocol has been utilized for the maintenance and differentiation of rat primary neurons (derived from E18 embryonic neural hippocampal tissue) and human NPC (derived from pluripotent stem cells), respectively and can be adapted for neural cells derived from other sources. Further optimization may be required depending on the cell type and application.

PDL Coating Procedure

For monolayer culture of neuronal cells, culture vessels must be pre-coated with substrate for cell attachment. Poly-D Lysine (PDL) (EMD Millipore, Catalog# A-003-E) is recommended.

1. Gently dilute PDL in sterile water, such as WFI (PN# 9309), to obtain a final working solution of 50 μg/mL.

2. Add the diluted PDL solution to the culture vessel at a ratio of 0.25 mL/cm^2. Refer to the table below for specific culture vessel instructions. Store the working PDL solution at 2-8°C for up to 1 month.

Culture Vessel	Surface Area (cm^2)	Volume of PDL solution (0.25mL/cm^2)
24-well plate	1.9	0.475 mL/well
12-well plate	3.8	0.95 mL/well
6-well plate	9.6	2.4 mL/well
T-25 Flask	25	6.25 mL
T-75 Flask	75	18.75 mL

3. Incubate culture vessels at 37°C for 2 hours to overnight in a 5% CO_2 incubator.

4. Aspirate the PDL solution from the culture vessel and wash three times with WFI. Make sure to rinse the culture vessel thoroughly as excess PDL can be toxic to the cells.

5. Leave the plate with lid off in a sterile biohazardous hood for 2-3 hours, or until each well is completely dry.

   NOTE: The dried plates may be used immediately or stored at 4°C, wrapped tightly with Parafilm® for up to 2 weeks.

Complete Medium Preparation

Prepare complete medium by supplementing the PRIME-XV Neural Basal Medium (PN# 91201) with 2 mM L-Glutamine Solution (PN# 9317) and a serum-free supplement such as PRIME-XV IS21 Supplement (50X) (PN# 91142) or N-2 Supplement (Thermo Fisher Scientific, Catalog# 17502). To prepare 100 mL of complete medium:

1. Aseptically add 2.0 mL of PRIME-XV IS21 Supplement (50X) to 98.0 mL of PRIME-XV Neural Basal Medium.

2. Aseptically add 0.25 mL of 200 mM L-Glutamine Solution to a final concentration of 0.5 mM to the medium before use.

3. Store the complete medium in the dark at 2-8°C for up to two weeks.

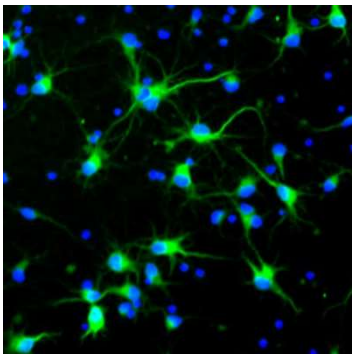
   NOTE: For larger volumes, increase the component amounts proportionally.

Plating Procedure for Neuronal Cell Maintenance and Differentiation

The following is a guideline for the maintenance of rat primary neurons derived from E18 embryonic neural hippocampal tissue and inducing differentiation of human neural progenitor cells (NPC) derived from pluripotent stem cells once ready for harvest and plating for differentiation.

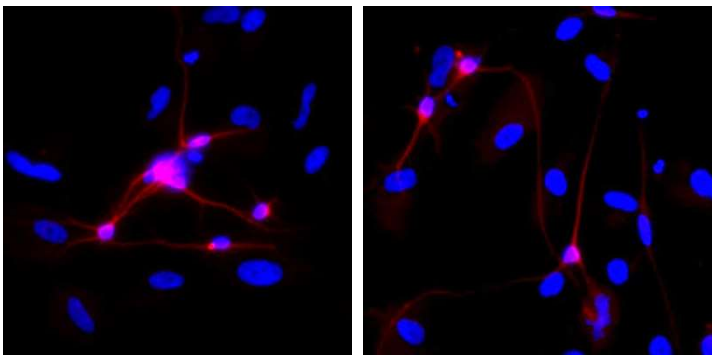
1. Prepare complete medium as described above and pre-warm 20 mL in a 37°C water bath.
2. Seed the cells in a pre-coated culture vessel (see PDL Coating Procedure) at a density of 0.8-1.25x10^5 cells/cm^2 (for rat hippocampal neurons) and 0.5-1x10^5 cells/cm^2 (for iPSC-derived human NPC) in an appropriate volume of pre-warmed complete medium.
3. Incubate at 37°C and 5% CO_2 humidified incubator.
4. Replace with fresh, pre-warmed complete medium every 3-4 days.
5. Observe cells regularly (2-3 times per week) to assess survival and viability.
6. At the end of culture period, cells can be processed for immunocytochemistry or other applications.

Sample Data



PRIME-XV Neural Basal Medium maintains primary rat hippocampal neurons.
Rat primary E18 embryonic hippocampal neurons were cultured in PRIME-XV Neural Basal Medium supplemented with PRIME-XV IS21 Supplement but no mitogens for 6 days. The cells were labelled with mouse anti-MAP2 (Thermo Scientific, Clone M13) to detect neurons (green). The nuclei were counterstained with DAPI (blue). Immunofluorescence images were taken at 20X magnification.

A **B**



PRIME-XV Neural Basal Medium retains neuronal differentiation potential of human neural progenitor cells.
iPSC-derived human NPC were cultured in PRIME-XV Neural Basal Medium supplemented with PRIME-XV IS21 Supplement. The differentiated cells were labeled with **(A)** mouse anti-MAP2 (Thermo Fisher Scientific, Clone M13) and **(B)** mouse neuron-specific anti-beta III Tubulin (R&D Systems, Clone Tuj1) to detect neurons (red). The nuclei were counterstained with DAPI (blue). Immunofluorescence images were taken at 40X magnification.

Related Products

Catalog #	Product	Size
91142	IS21 Supplement (50X)	10 mL liquid
9317	L-Glutamine Solution (200mM)	100 mL, 500 mL liquid
91139	PRIME-XV FreezIS Solution	10 mL, 100 mL liquid
9228	HBSS without calcium and magnesium	100 mL, 500 mL liquid
9240	PBS without calcium and magnesium	100 mL, 500 mL, 1 L 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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