PRIME-XV IS21 SUPPLEMENT (50X)

Catalog #	Product	Size
91142	PRIME-XV IS21 Supplement	10 mL liquid

Intended Use

For research or further manufacturing use only.

Product Description

PRIME-XV IS21 Supplement (50X) is a serum-free supplement optimized for use with PRIME-XV Neural Basal Medium to support maintenance and long-term culture of neuronal cells. This supplement is supplied as a 50X concentrated solution and should be diluted to 1X in basal medium before use. This supplement 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 supplement 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 IS21 Supplement (50X) can be aliquoted and stored at or below -10°C in a manual defrost freezer for up to 6 months. Repeated freeze/thaw cycles should be avoided. When ready to use, thaw this supplement overnight at 4°C in the dark. Once combined with basal medium, the complete medium can 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 IS21 Supplement (50X) 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

Neuronal Long-term Culture

The following protocol has been utilized for long-term culture of rat primary neurons (derived from E18 embryonic neural hippocampal tissue). 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 (0.25 mL/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 diluting PRIME-XV IS21 Supplement (50X) (PN# 91142) 50-fold to 1X into appropriate neural basal medium, such as PRIME-XV Neural Basal Medium (PN# 91201) with 2 mM L-Glutamine Solution (PN# 9317). 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.

Recovery of Cryopreserved Cells

The following is a guideline based on the cryopreservation of rat primary neurons. 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 PDL Coating Procedure) at a seeding density of 0.8-1.25x10^5 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.

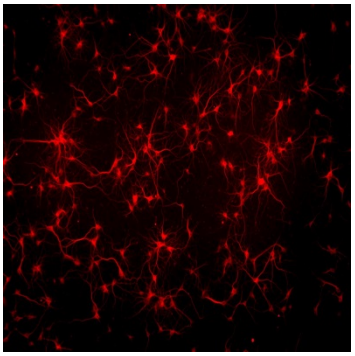
7. 24 hours post-thaw, remove half of the spent medium from the culture vessel and replace with an equal amount of pre-warmed culture medium.

8. Change the medium in the culture vessel with fresh pre-warmed complete medium every 4 days.

9. Observe the cells regularly to assess survival and viability of neurons.

10. At the end of the culture period, the cells can be processed for immunocytochemistry or other applications.

Sample Data



PRIME-XV IS21 Supplement (50X) supports maintainence of primary rat hippocampal neurons.
Rat primary E18 embryonic hippocampal neurons were cultured in PRIME-XV Neural Basal Medium supplemented with PRIME-XV IS21 Supplement for 14 days. The cells were labelled with mouse anti-MAP2 (Thermo Fisher Scientific, Clone M13) to detect neurons (red). Immunofluorescence images were taken at 10X 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.

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 (see Complete Medium Preparation) 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.

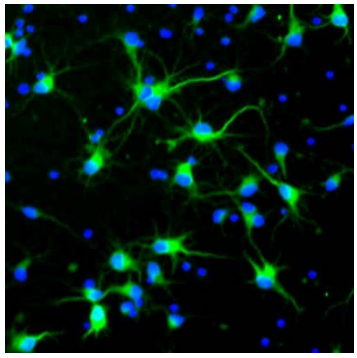
3. Incubate at 37°C and 5% CO_2 humidified incubator.

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.

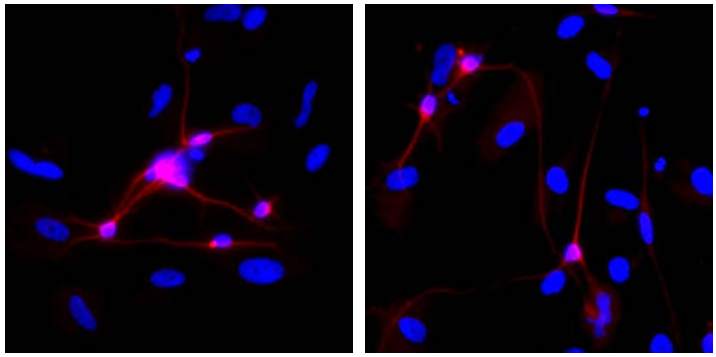
6. At the end of culture period, cells can be processed for immunocytochemistry or other applications.

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 Fisher 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
91201	PRIME-XV Neural Basal Medium	500 mL liquid
91131	PRIME-XV NPC Expansion XSFM	250 mL liquid
95108	Recombinant Human EGF ACF	100 µg
95109	Recombinant Human FGF-basic 154 ACF	10 µg
9317	L-Glutamine Solution (200 mM)	100 mL, 500 mL liquid
9309	Water for Injection (WFI) Quality Water	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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