PRIME-XV MSC Expansion XSFM

Catalog #	Product	Size
91149	PRIME-XV MSC Expansion XSFM	250 mL and 1 L liquid

Intended Use

This product is for research use or further manufacturing use only. This product is not for injection or diagnostic procedures. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Product Description

PRIME-XV MSC Expansion XSFM is a serum-free and xeno-free complete medium optimized for the maintenance and expansion of purified human MSCs. This product does not contain antibiotics. PRIME-XV MSC Expansion XSFM is used in the maintenance and expansion of purified or enriched human mesenchymal stem/stromal cells (MSCs) under serum-free and xeno-free culture conditions. This medium is ready to use. It may also be used with additional cytokine/growth factors for desired application.

Shipping

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

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.

Storage Instructions and Stability

Upon receipt, store the medium at or below -10°C in a manual defrost freezer. Unopened medium is stable for 24 months from date of manufacture, as indicated on label, when stored at or below -10°C in a manual defrost freezer. PRIME-XV MSC 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 MSC Expansion XSFM should be used within one week when stored at 2-8°C and protected from light. Not validated for use beyond the unopened expiry shelf life. Repeated freeze-thaw cycles should be avoided.

NOTE: As an enriched media the presence of precipitates may occur over time. The presence of precipitates has not been shown to cause any detrimental on effect on product performance. If desired, the media can be aliquoted into sterile tubes and centrifuged for 5 minutes at 300 g before use.

Precautions

Results may vary due to variations among human MSCs derived from different donors.

This product contains components 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

The following protocol is optimized for expansion of MSCs derived from adipose, bone marrow, and umbilical cord in two dimensional culture vessels.

Coating Procedure

NOTE: To grow MSCs in PRIME-XV MSC Expansion XSFM, tissue culture plates or flasks have to be pre-coated with substrate for cell attachment. It is highly recommended to use Cellnest (FISI, Catalog ID 1063967) to coat culture surfaces for consistency. Other types of matrix substrate (PRIME-XV MatrIS F, FISI, Catalog ID 31001 or PRIME-XV Human Fibronectin, FISI, Catalog ID 31002) may be used, where types and amounts of matrix protein are dependent on the experimental design of each individual investigator.

1. Prepare 0.5% Cellnest solution by gently adding 5 mL sterile water to the lyophilized Cellnest (FISI, Catalog ID 1063967) in the vial to make a concentration of 5 mg/mL.
2. Reassemble the cap on the vial and incubate the 0.5% Cellnest solution at 37°C for 10 minutes to fully dissolve the Cellnest.
3. Filter the 0.5% Cellnest solution through an 0.22 µm regenerated cellulose or PES filter to ensure sterility and bring the filter sterilized solution in a new sterile 15 ml centrifuge tube. Please use small diameter filters (13 mm) to minimize volume loss during filtration (e.g. Nalgene, ID 720-1320). This 0.5% Cellnest solution can be stored at 2-8°C for six months or at -20°C for twelve months.
4. Dilute the 0.5% stock solution in PBS (FISI, Catalog ID 9236) to a final concentration of 20 µg/cm2. Refer to the table below for respective culture vessel.

Culture Vessel	Finale Volume (mL)	PBS (mL)	Filter sterile 0.5% Cellnest (uL)	Cellnest per cm^2	[Cellnest] ug/ml	[Cellnest] mM
6-well (9.6 cm^2 per well)	2.0	1.96	38.4	20	96	1.9
6-well plate (57.6 cm^2 per plate)	12.0	11.77	230.4	20	96	1.9
T-25 Flask (25 cm^2 per flask)	5.2	5.10	100.0	20	96	1.9
T-75 Flask (75 cm^2 per flask)	15.5	15.20	300.0	20	97	1.9

5. Add the diluted coating solution to the desired culture vessel.
6. Incubate the plate at one of the following conditions. The culture vessel must be sealed with Parafilm® to avoid drying if stored at 2-8°C overnight. It is recommended to coat culture vessels the day of use or the day before use.

a. One hour at 37±2°C

b. Three hours at 15-30°C

c. Overnight at 2-8°C

7. Aspirate out and discard the Cellnest solution from culture vessels right before the addition of cells.

Recovery of Cryopreserved Human MSCs

1. Pre-coat the tissue culture vessel with diluted Cellnest in PBS to a final concentration 20 µg/cm^2 following the coating protocol.
2. Pre-warm PRIME-XV MSC Expansion XSFM to 37°C.
3. Rapidly thaw frozen vial of cells in a 37°C water bath.
4. Pipet the entire contents of the cryovial into a 15 mL conical tube. Carefully add 5 to 10 mL of pre-warmed PRIME-XV MSC Expansion XSFM at an approximate rate of 3 to 5 drops per 10 seconds and gently swirl after every addition.
5. Transfer the entire contents of the conical tube into a Cellnest coated tissue culture vessel.
6. Incubate the cells at 37°C in a humidified atmosphere of 5% CO_2 in air.
7. Aspirate off media and feed the cells with pre-warmed PRIME-XV MSC Expansion XSFM 24 hours post-thaw.
8. Every 2 days, remove and discard spent media, and feed the cells with pre-warmed PRIME-XV MSC Expansion XSFM.
9. Subculture when cells reach 70-80% confluence. Do not allow the cultures to become totally or over confluent. Subculturing under suboptimal conditions may affect product performance.

Subculturing Human MSCs in PRIME-XV MSC Expansion XSFM

1. Pre-coat the tissue culture vessel diluted Cellnest in PBS to a final concentration 20 µg/cm2 following the coating protocol.
2. Pre-warm PRIME-XV MSC Expansion XSFM to 37°C.
3. Remove spent media from T75 flask culture (for example) and gently rinse cells once with 10 mL of PBS (FISI, Catalog ID 9240) for each T75 flask.
4. Add 5 mL of room temperature TrypLE Express to each T75 flask, and tilt the flask in all directions to disperse TrypLE Express evenly over the cells.
5. Incubate the cells at 37°C, 5% CO_2 incubator. Monitoring periodically for cell detachment by observing the cells under the microscope. Cells will start to round and detach. Tap the side of the flask to aid the detachment of the cells and return culture to the incubator. Repeat the above process until at least 90% of cells are fully detached. This process takes approximately 5 – 10 minutes.
6. Add 5 mL of PRIME-XV MSC Expansion XSFM to the flask. Disperse the cells by pipetting the media over the entire growing surface of the flask, and transfer the contents to a 15 mL conical tube.
7. Centrifuge cells down at 400 x g for 5 minutes. Aspirate off supernatant.
8. Resuspend the cell pellet in a small amount of pre-warmed PRIME-XV MSC Expansion XSFM and count the cells with a cell counter.
9. Resuspend 4.5 – 5.0 x 10^5 cells into 20 mL of pre-warmed PRIME-XV MSC Expansion XSFM for each PRIME-XV MatrIS F coated T75 flask.

Note: It is recommended to seed cells at approximately 6000 cells/cm^2/0.2-0.3 mL of media for 2-dimensional pre-coated culture vessels.

10. Gently aspirate PRIME-XV MatrIS F solution from the flask, and slowly add the cell suspension to a T75 flask. Avoid scraping the coated surface when aspirating off PRIME-XV MatrIS F solution. Incubate the cells at 37°C and 5% CO_2 in a humidified atmosphere.
11. Remove and discard spent media, and feed the cells with pre-warmed PRIME-XV MSC Expansion XSFM every 2 days.

Data

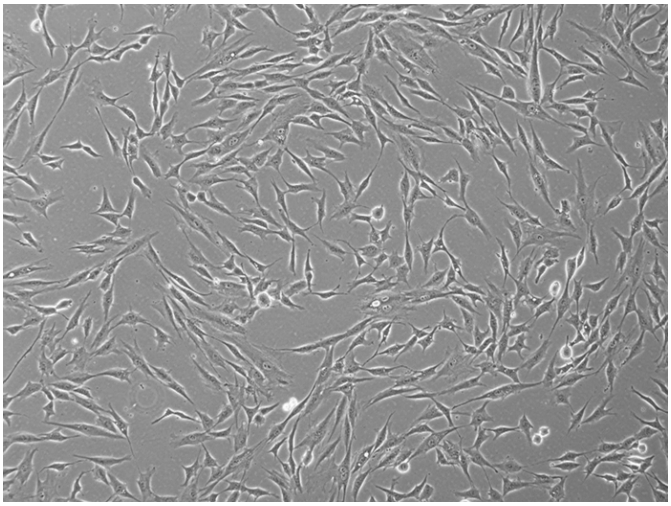

Figure 1. Morphology of human adipose-derived MSCs cultured in PRIME-XV MSC Expansion XSFM on PRIME-XV Human Fibronectin. MSCs were plated at 6,000 cells/cm^2 and morphology was observed after prolonged passaging in PRIME-XV MSC Expansion XSFM. Attachment and morphology were typically observed three to four days after passaging. Images were taken at 10X magnification.

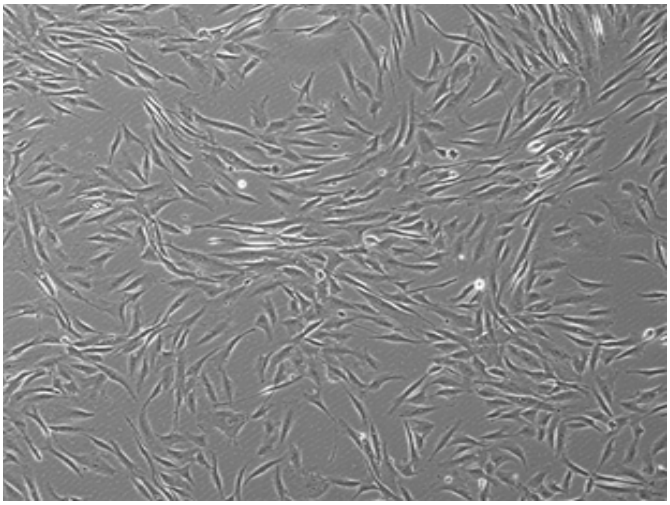

Figure 2. Morphology of human bone marrow-derived MSCs cultured in PRIME-XV MSC Expansion XSFM on PRIME-XV Human Fibronectin. MSCs were plated at 6,000 cells/cm^2 and morphology was observed after prolonged passaging in PRIME-XV MSC Expansion XSFM. Attachment and morphology were typically observed four to five days after passaging. Images were taken at 10X magnification.

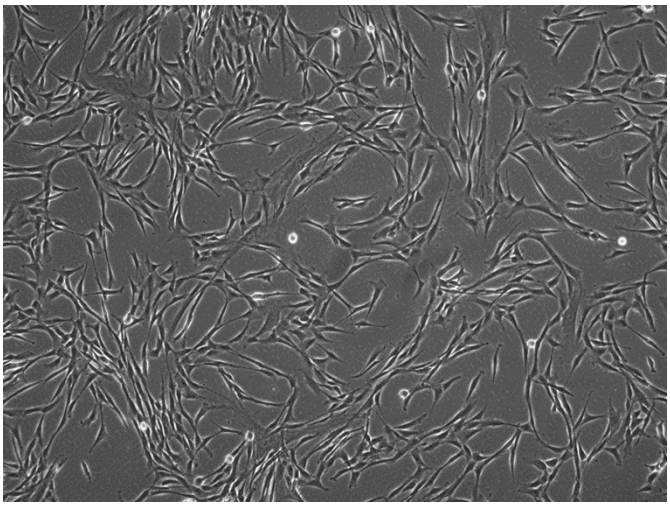

Figure 3. Morphology of human umbilical cord-derived MSCs cultured in PRIME-XV MSC Expansion XSFM on PRIME-XV Human Fibronectin. MSCs were plated at 6,000 cells/cm^2 and morphology was observed after prolonged passaging in PRIME-XV MSC Expansion XSFM. Attachment and morphology is typically observed three to four days after passaging. Images were taken at 10X magnification.

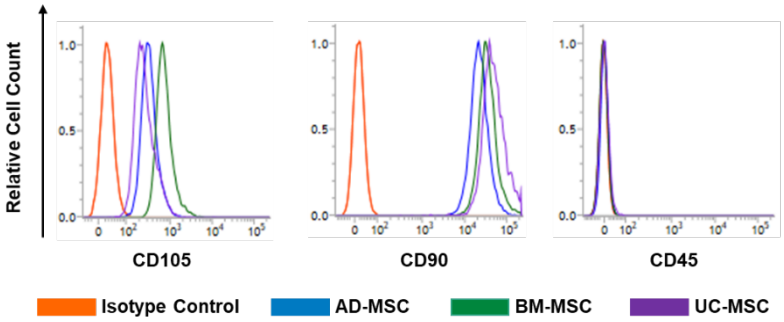

Figure 4. Phenotypic analysis of human adipose (AD-MSC), bone marrow-derived MSCs (BM-MSC), and umbilical cord-derived MSCs (UC-MSC) expanded in PRIME-XV MSC EXPANSION XSFM. MSCs were expanded for 3 passages. Phenotypic analysis was analyzed by flow cytometry. Cells were positive for CD105 and CD90 cell surface markers but lacked CD45 expression. For each antibody, isotype matched controls were also included.

Related Products

Catalog #	Product	Size
91140	PRIME-XV Stem FreezIS DMSO-Free	10 mL, and 100 mL liquid
91132	PRIME-XV Osteogenic Differentiation SFM	100 mL liquid
91137	PRIME-XV Adipogenic Differentiation SFM	100 mL liquid
91138	PRIME-XV Chondrogenic Differentiation XSFM	100 mL liquid
1063967	Cellnest	25 mg lyophilized powder
31002	PRIME-XV Human Fibronectin	1 mg liquid
31001	PRIME-XV MatrIS F	200 µL 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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