

PRIME-XV[®] MSC Expansion SFM

PRIME-XV[®] MSC Expansion SFM is a serum-free complete medium optimized for the maintenance and expansion of purified human MSCs. This product does not contain antibiotics.

Catalog #	Product	Format	Available Package Sizes
91135	PRIME-XV [®] MSC Expansion SFM	Liquid	250 mL and 1 L Additional package sizes are available at request

Intended Use

PRIME-XV[®] MSC Expansion SFM is intended for use in the maintenance and expansion of purified or enriched human mesenchymal stromal/ stem cells (MSCs) under serum-free culture conditions. This medium is ready to use or it may be used with additional cytokine/growth factors for the desired application.

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 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 24 months from date of manufacture, as indicated on label, when stored at or below -10°C in a manual defrost freezer. When ready to use, thaw this medium overnight at 2-8°C in the dark. PRIME-XV MSC Expansion SFM 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 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 and Warnings

For research use or further manufacturing use only. Not for injection or 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 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 bone marrow in two dimensional culture vessels.

Coating Procedures

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

- 1 Gently add PRIME-XV MatrIS F in PBS (Irvine Scientific, Catalog # 9236) to a final concentration of 5 $\mu\text{g}/\text{mL}$.
- 2 Add the diluted coating PRIME-XV MatrIS F solution to culture vessel at a final volume per surface area of 0.08 mL/cm^2 . Refer to the table for respective culture vessel:

Culture Vessel	Surface Area (cm ²)	Volume of 5 $\mu\text{g}/\text{mL}$ MatrIS F
6-well plate	9.6	768 μL per well
T-25 flask	25	2 mL per flask
T-75 flask	75	6 mL per flask

- 3 Incubate culture vessels at room temperature for 3 hours or overnight at 2 – 8°C. 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.
- 4 Aspirate out and discard diluted PRIME-XV MatrIS F solution from culture vessels before the addition of cells.

Recovery of Cryopreserved Human MSCs

- 1 Pre-coat the tissue culture vessel with 5 $\mu\text{g}/\text{mL}$ of PRIME-XV MatrIS F in PBS for 3 hours at room temperature or overnight at 2 – 8°C. See Coating Procedures.
- 2 Pre-warm PRIME-XV MSC Expansion SFM 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 SFM 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 PRIME-XV MatrIS F coated tissue culture vessel.
- 6 Incubate the cells at 37°C in a humidified atmosphere of 5% CO₂ in air.
- 7 Aspirate off media and feed the cells with pre-warmed PRIME-XV MSC Expansion SFM 24 hours post-thaw.
- 8 Every 2 days remove and discard spent media, and feed the cells with pre-warmed PRIME-XV MSC Expansion SFM.
- 9 Subculture when cells reach 80-90% confluence. Do not allow the cultures become totally or over confluent. Subculturing under suboptimal conditions may affect product performance.

Subculturing Human MSCs in PRIME-XV MSC Expansion SFM

1. Pre-coat the tissue culture vessel with 5 µg/mL of PRIME-XV MatrIS F in PBS for 3 hours at room temperature or overnight at 2 – 8°C. See Coating Procedures.
2. Pre-warm PRIME-XV MSC Expansion SFM to 37°C.
3. Remove spent media from T75 flask culture and gently rinse cells once with 10 mL of PBS (Irvine Scientific, Catalog # 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% CO₂ 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 SFM 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 SFM and count the cells with a cell counter.
9. Resuspend 4.5 - 5.0 x 10⁵ cells into 20 mL of pre-warmed PRIME-XV MSC Expansion SFM for each PRIME-XV MatrIS F coated T75 flask. Note: It is recommended to seed cells at approximately 6000 cells/cm²/0.2-0.3 mL of media for 2-dimensional pre-coated culture vessels.
10. Gently aspirate off 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% CO₂ in a humidified atmosphere.
11. Remove and discard spent media, and feed the cells with pre-warmed PRIME-XV MSC Expansion SFM every 2 days.

Data

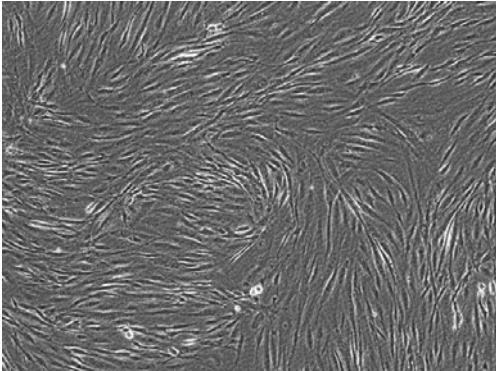


Figure 1. Morphology of human MSCs derived from bone marrow cultured with PRIME-XV MSC Expansion SFM on PRIME-XV Matris F.

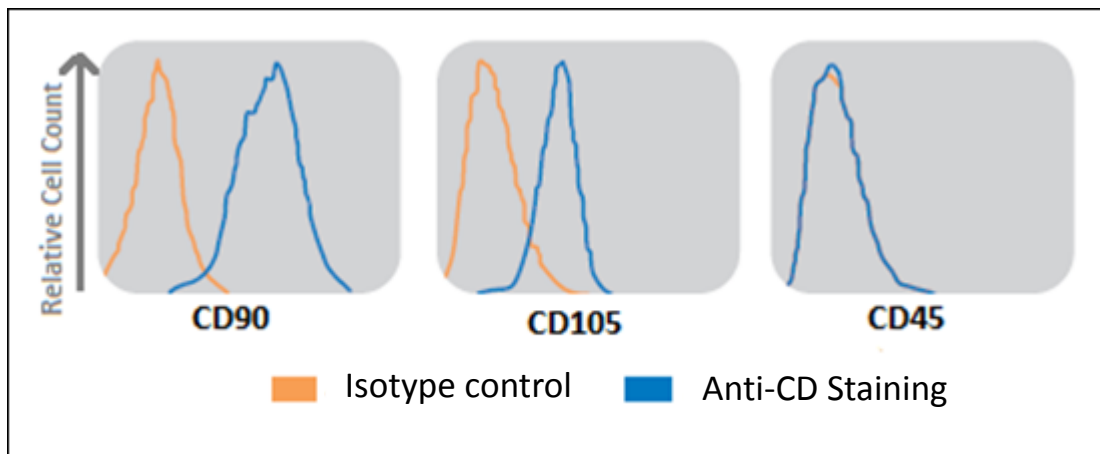


Figure 2. Phenotypic analysis of human bone marrow-derived MSCs expanded in PRIME-XV MSC Expansion SFM for 3 passages were analyzed by flow cytometry. Cells were positive for CD105 and CD90 cell surface markers but lacked CD45 expression. For each antibody, isotype-matched controls were also included.

Related Products

Catalog #	Product	Size
31001	PRIME-XV Matris F	100 µg
31002	PRIME-XV Human Fibronectin	100 mg
91139	PRIME-XV FreezIS	10 mL
91140	PRIME-XV MSC FreezIS DMSO-Free	10 mL

Technical Support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: tmrequest@irvinesci.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

Irvine Scientific

2511 Daimler Street, Santa Ana, California 92705 USA
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com

© 2017 Irvine Scientific. All rights reserved. Irvine Scientific and PRIME-XV are registered trademarks of Irvine Scientific Sales Company, Inc. All other trademarks are the property of their respective owners. P/N 40967 Rev.4

