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.

Precautions

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

Components for this product have been sourced following the guidelines of EP 5.2.12, “Raw Materials of Biological Origin for the Production of Cell-Based and Gene Therapy Medicinal Products”. This product contains components derived from human plasma, which are USP- and EP-grade, manufactured following Good Manufacturing Practices and have been tested and found negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) using FDA-

licensed kits. However, since no test method offers complete assurance that products of human origin are noninfectious, handle all human source material as if potentially infectious using universal precautions.

Directions for Use

PROTOCOL FOR MSC EXPANSION IN 2D CULTURE VESSELS

The following protocol is optimized for the expansion of human mesenchymal stem cells (hMSCs) 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 Human Fibronectin, FISI, and 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 ten minutes to fully dissolve the Cellnest.
3. Filter the 0.5% Cellnest solution through a 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/cm^2. 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 three to five drops per ten 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 two 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/cm^2 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 five to ten 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 400xg 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 4.5–5.0x10^5 cells into 20 mL of pre-warmed PRIME-XV MSC Expansion XSFM for each 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 solution from the flask, and slowly add the cell suspension to a T75 flask. Avoid scraping the coated surface when aspirating off 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 two days.

PROTOCOL FOR MSC EXPANSION IN QUANTUM BIOREACTOR

PRIME-XV MSC XSFM 91149 in 1L bottles may be aseptically transferred to Quantum-compatible bags and used for hMSC expansion in the Quantum. A typical round of MSC expansion over seven days requires five liters of media. Due to the short shelf life of 91149 post-thaw, the bottles must be thawed and Quantum-compatible bag prepared no more than a day before initiating the expansion. Re-freezing bagged media is not recommended. Media function may be negatively affected if passed through a filter prior to bagging. Ensuring sterility of bagged media is critical for optimal cell expansion.

Day (-1): Prepare cell expansion set and coat bioreactor

NOTE: The Quantum Cell Expansion System Operator's Manual (QCESOM) refers to the attachment of cell, media, or waste bags to lines. This is facilitated with a sterile tubing welder that is compatible with Pellethane (thermoplastic polyurethane elastomer). Please refer to the appropriate welder's instruction manual for proper operation and handling. The following protocol utilizes the TSCD-Q Sterile Tubing Welder from Terumo BCT. The corresponding manual, TSCD-Q Sterile Tubing Welder System Operator's Manual (TSTWSOM), contains the necessary information for safe operation.

1. If thawing cells, let rest overnight in culture flask and complete culture media at 37˚C, 5% CO_2.
2. Load cell expansion set (QCESOM 7-2)
3. Attach bag with PBS to Wash line (TSTWSOM 3-2).
4. Prime cell expansion set (QCESOM 7-11)
5. Separate the tube lines (QCESOM 7-12)
6. Prepare 100 mL of coating solution with PBS and 5 mg of fibronectin.
    a. Load coating solution and at least 40 mL of air into cell inlet bag and attach to reagent line.
    b. Set up and run the Coat Bioreactor program (QCESOM 8-8). Use the following settings:

	Step 1	Step 2	Step 3
IC inlet	Reagent	Wash	None
IC inlet rate (mL/min)	10	10	0
IC circ. rate (mL/min)	100	100	20
EC inlet	None	None	Wash
EC inlet rate (mL/min)	0	0	0.1
EC circ. rate (mL/min)	30	30	30
Outlet	EC outlet	EC outlet	EC outlet
Rocker	Stationary (0˚)	Stationary (0˚)	Stationary (0˚)
Stop condition	Empty bag	IC Volume (22 mL)	Manual (overnight)

Day 0: Prepare IC media and load cells

7. Attach the PRIME-XV MSC XSFM media bag to IC line and run IC/EC Washout (QCESOM Section 9-5). Use the following settings:

	Step 1
IC inlet	IC media
IC inlet rate (mL/min)	100
IC circ. rate (mL/min)	-17
EC inlet	IC media
EC inlet rate (mL/min)	148
EC circ. rate (mL/min)	-1.7
Outlet	IC & EC outlet
Rocker	In motion (-90˚, 180˚, 1 sec)
Stop condition	Exchange (2.5 IC, 2.5 EC)

8. Cover the complete medium bag with aluminum foil to protect from light. Condition media (QCESOM Section 8-6) with the gas supply on. Use the following settings:

	Step 1	Step 2
IC inlet	None	None
IC inlet rate (mL/min)	0	0
IC circ. rate (mL/min)	100	100
EC inlet	IC media	IC media
EC inlet rate (mL/min)	0.1	0.1
EC circ. rate (mL/min)	250	30
Outlet	EC outlet	EC outlet
Rocker	Stationary (0˚)	Stationary (0˚)
Stop condition	Time (10 min)	Manual (50-230 min)

9. Dissociate cells from culture flask(s), perform cell count, and save a sample for flow cytometry
10. Load $25x10^6$ cells, 100 mL IC media, and at least 40 mL air into a cell inlet bag. Attach to cell line.
11. Ensure that appropriate bags are attached to their corresponding lines (IC media, Cell, Wash) and replace the waste bag with a fresh one.
    a. Replace waste bags and IC media bags as needed throughout culture by pausing the program for the duration of the replacement process.
12. Take a sample of media from the EC sample port for baseline metabolite readings (QCESOM Section 5-7).

13. Set up and run the Load Cells with Uniform Suspension program (QCESOM Section 10-9). Use the following settings:

	Step 1	Step 2	Step 3
IC inlet	Cell	IC media	None
IC inlet rate (mL/min)	25	25	0
IC circ. rate (mL/min)	150	150	200
EC inlet	None	None	None
EC inlet rate (mL/min)	0	0	0
EC circ. rate (mL/min)	30	30	30
Outlet	EC outlet	EC outlet	EC outlet
Rocker	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)
Stop condition	Empty bag	IC volume (47 mL)	Time (2 min)

14. Set up and run the Attach Cells program (QCESOM Section 10-7). Use the following settings:

	Step 1
IC inlet	None
IC inlet rate (mL/min)	0
IC circ. rate (mL/min)	0
EC inlet	IC media
EC inlet rate (mL/min)	0.1
EC circ. rate (mL/min)	30
Outlet	EC outlet
Rocker	Stationary (0˚)
Stop condition	Manual (1440 min)

Day 1: Begin feeding culture

15. Dissociate cells from culture flask(s), perform cell count, and save a sample for flow cytometry

	Feed (Days 1-7)
IC inlet	IC media
IC inlet rate (mL/min)	0.1 - Variable
IC circ. rate (mL/min)	20
EC inlet	None
EC inlet rate (mL/min)	0
EC circ. rate (mL/min)	30
Outlet	IC outlet
Rocker	Stationary (0˚)
Stop condition	Manual

16. Collect daily samples of EC media from the EC sampling port for metabolite analysis, taking note of the time these samples were taken (QCESOM Section 5-7). Adjust IC inlet rate as necessary to maintain appropriate glucose and lactate levels in culture, keeping in mind media consumption and waste bag fill rates. This continues for the duration of the culture.

Harvest and analyze cells

17. Prepare a cell inlet bag with 200 mL trypsin solution and 40 mL air. Attach to Reagent line.
18. Set up and run the Release Adherent Cells and Harvest program (QCESOM 12-5). Use the following settings:

	Step 1	Step 2	Step 3	Step 4	Step 5
IC inlet	Wash	Reagent	Wash	None	EC media
IC inlet rate (mL/min)	100	50	50	0	400
IC circ. rate (mL/min)	-17	300	300	300	-70
EC inlet	Wash	None	None	None	EC media
EC inlet rate (mL/min)	148	0	0	0	60
EC circ. rate (mL/min)	-1.7	30	30	30	30
Outlet	IC & EC outlet	EC outlet	EC outlet	EC outlet	Harvest
Rocker	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)
Stop condition	Exchange (2.5 IC, 2.5 EC)	Empty bag	IC volume (22 mL)	Time (8* min)	IC Volume (378 mL)

*Time required when using 0.25% Trypsin. When using TrypLE™, twenty minutes is recommended.

19. Detach cell harvest bag and run cell counts, spent media analysis, and flow cytometry.

Data

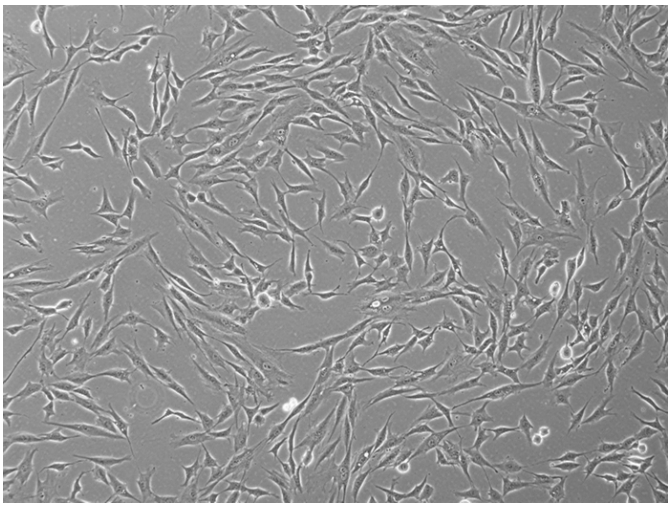

Figure 1. Morphology of human adipose-derived MSCs cultured in PRIME-XV MSC Expansion XSFM on PRIME-XV Human Fibronectin. MSCs were plated at 6,000 cells/cm^2 and morphology was observed after prolonged passaging in PRIME-XV MSC Expansion XSFM. Attachment and morphology were typically observed three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

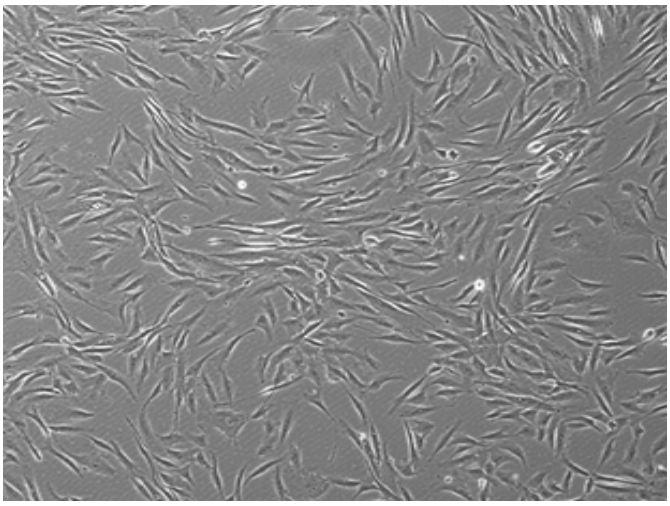

Figure 2. Morphology of human bone marrow-derived MSCs cultured in PRIME-XV MSC Expansion XSFM on PRIME-XV Human Fibronectin. MSCs were plated at 6,000 cells/cm^2 and morphology was observed after prolonged passaging in PRIME-XV MSC Expansion XSFM. Attachment and morphology were typically observed four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

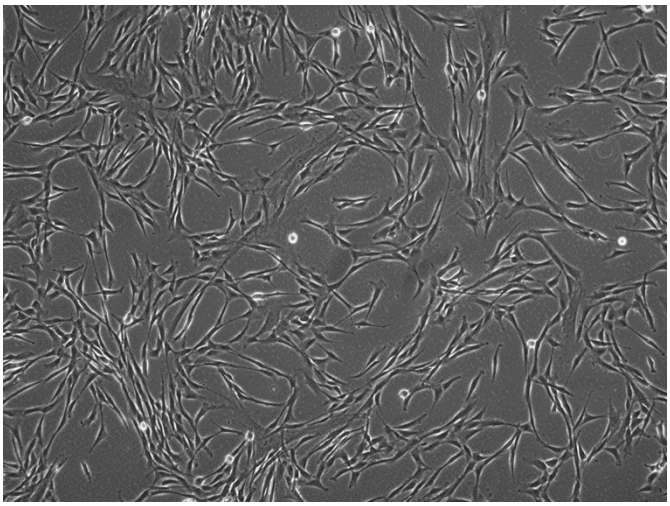

Figure 3. Morphology of human umbilical cord-derived MSCs cultured in PRIME-XV MSC Expansion XSFM on PRIME-XV Human Fibronectin. MSCs were plated at 6,000 cells/cm^2 and morphology was observed after prolonged passaging in PRIME-XV MSC Expansion XSFM. Attachment and morphology is typically observed three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

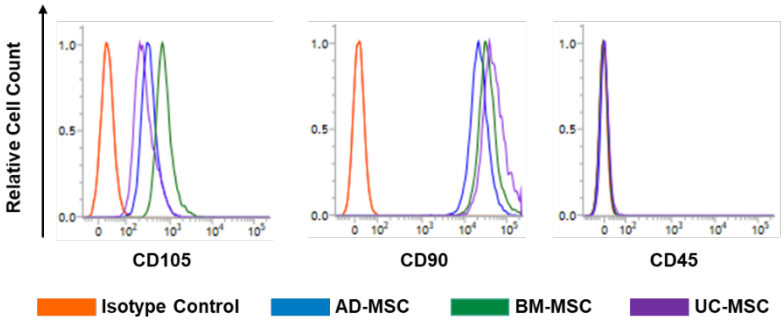

Figure 4. Phenotypic analysis of human adipose (AD-MSC), bone marrow-derived MSCs (BM-MSC), and umbilical cord-derived MSCs (UC-MSC) expanded in PRIME-XV MSC EXPANSION XSFM. MSCs were expanded for three passages. Phenotypic analysis was analyzed by flow cytometry. Cells were positive for CD105 and CD90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

Related Products

Catalog #	Product	Size
91140	PRIME-XV FreezIS DMSO-Free	10 mL, and 100 mL liquid
91139	PRIME-XV FreezIS	10 mL, and 100 mL liquid
91137	PRIME-XV Adipogenic Differentiation SFM	100 mL liquid
91138	PRIME-XV Chondrogenic Differentiation XSFM	100 mL liquid
91135	PRIME-XV MSC Expansion SFM	250 mL and 1 L liquid
31002	PRIME-XV Human Fibronectin	1 mg liquid
91149DC	PRIME-XV MSC XSFM Dual Component with Phenol Red	Kit of 5L MSC basal and 10ml frozen supplement in bag
91214DC	PRIME-XV MSC XSFM Dual Component without Phenol Red	Kit of 5L MSC basal and 10ml frozen supplement in bag

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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Effective Date: