PRIME-XV MSC XSFM Dual Component Kit with Phenol Red

Catalog #	Product	Size
91149DC	PRIME-XV MSC XSFM Dual Component Kit with Phenol Red	10 mL frozen supplement bag and 5 L liquid bag

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 XSFM Dual Component Kit with Phenol Red, 91149DC is a dual-component serum-free and xeno-free 5L basal medium bag and a frozen supplement bag. When combined, the formulation is optimized for the maintenance and expansion of purified or enriched human mesenchymal stromal/stem cells (MSCs) in the Terumo Quantum or Quantum Flex Bioreactor, or other compatible bioreactors, under serum-free and xeno-free culture conditions. This product does not contain antibiotics. This medium is provided as a basal medium and supplement and should be mixed prior to use. It may also be used with additional cytokine/growth factors for desired application.

Shipping

PRIME-XV MSC XSFM BASE with Phenol Red, 91235-5L-60494 is shipped at 2-8°C. PRIME-XV MSC XSFM SUPPLEMENT 91236-10mL-60493 is shipped in dry ice at -20°C. Upon receipt, store the MSC basal medium at 2-8°C and supplement at -20°C.

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 MSC basal medium at 2-8°C and supplement at -20°C. Unopened basal media and frozen supplement are stable for 12 months from date of manufacture, as indicated on label, when stored at their respective temperatures. After mixing the 2 components, the complete media is equivalent to catalog part number 91149 PRIME-XV MSC Expansion XSFM and can be aseptically aliquoted and stored at or below -20°C in a manual defrost freezer for up to 3 months. Freezing media directly in the bag is not recommended. When ready to use, thaw the supplement bag overnight at 2-8°C in the dark, and bring the basal bag to room temperature overnight in the dark. Once combined with the supplement, PRIME-XV MSC XSFM Dual Component Kit with Phenol Red, 91149DC 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, sourced from FDA-licensed and inspected donation centers and tested, and found negative for, HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). 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

The following protocol is optimized for the expansion of human mesenchymal stromal/stem cells (hMSCs) derived from adipose, bone marrow, and umbilical cord in the Quantum, a Terumo BCT hollow fiber bioreactor, using the PRIME-XV MSC XSFM Dual Component Kit with Phenol Red, 91149DC.

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.

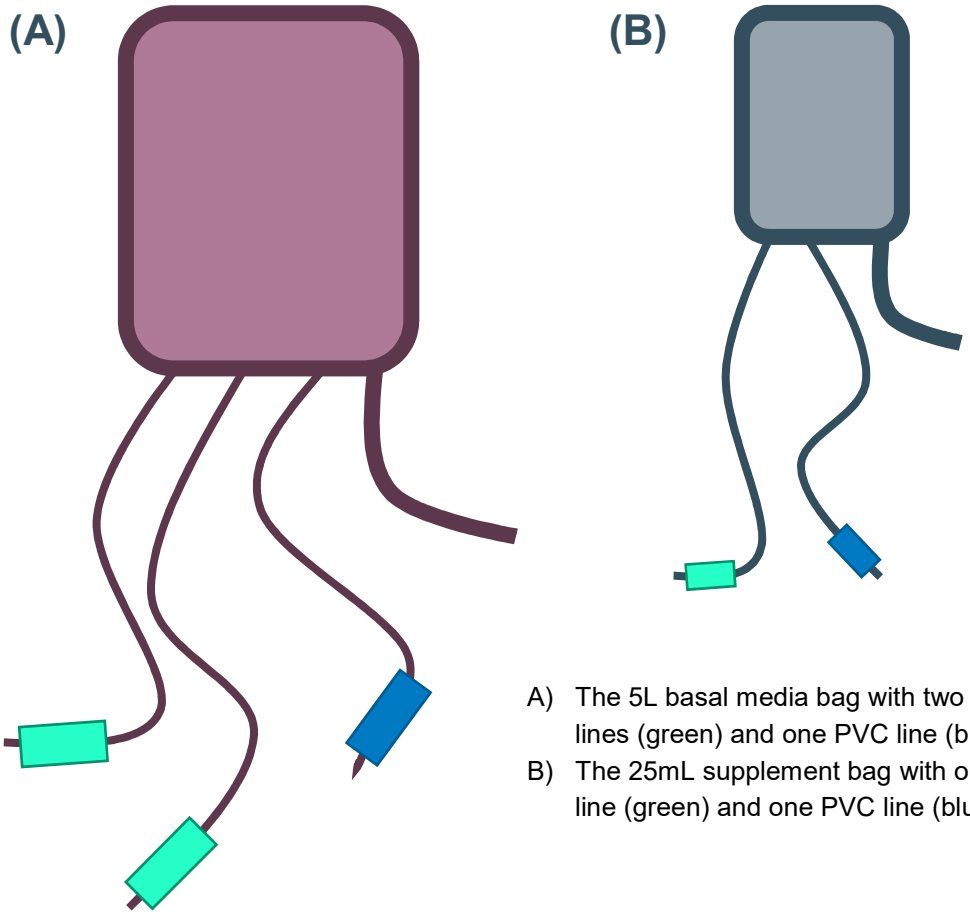
1. If thawing cells, let rest overnight in culture flask and complete culture media at 37˚C, 5% CO_2.
2. Load cell expansion set (QCESOM 7-2).
3. Attach bag with PBS to Wash line (TSTWSOM 3-2).
4. Prime cell expansion set (QCESOM 7-11).
5. Separate the tube lines (QCESOM 7-12).
6. Prepare 100 mL of coating 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. Connect and mix basal medium bag with supplement bag
    a. Make sure the supplement bag is completely thawed
    b. Hang a bag of PRIME-XV MSC XSFM Base (+) Phenol Red (91235-5L-60494) on the Quantum rotating bag pole.
    c. Select the appropriate tubing to weld together on each bag. Each bag has two types of tubing, labeled with colored tape for convenience.
        i. Green-labeled tubing is made of Pellethane and may be welded using the TSCD-Q Sterile Tubing Welder. These lines are directly compatible with the cell expansion set and the ones that that FISI routinely uses.
        ii. Blue-labeled tubing is PVC and may be welded together using the TSCD-II Sterile Tubing Welder.
        iii. The PVC and Pellethane tubing are not compatible for welding to each other.
    d. Aseptically connect the compatible tubes from each bag together using the corresponding Sterile Tubing Welder.



A) The 5L basal media bag with two Pellethane lines (green) and one PVC line (blue)
B) The 25mL supplement bag with one Pellethane line (green) and one PVC line (blue)

e. Remove the attached tubes from the welder and inspect the weld. If the weld is correct, “pop” the seal by applying pressure and rolling it against a hard surface (TSTWSOM 3-6).

f. Allow the contents of the supplement bag to flow by lowering the basal medium bag while holding the supplement bag higher. Alternate the bags so that contents from one flow into the other, repeating 5-7 times. This ensures that any leftover supplement content in the tubes and supplement bag transfer completely to the basal medium bag.

8. Detach the supplement bag from the basal medium bag using a sterile sealer to create a 3-point seal in the middle of the tube. Be sure to fully empty the supplement bag into the basal bag prior to sealing. Carefully cut through the middle seal with scissors. Properly mix the contents of the bag by tilting it left and right ten times.

9. Attach IC 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)

10. 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)

11. Dissociate cells from culture flask(s), perform cell count, and save a sample for flow cytometry
12. Load $25x10^6$ cells, 100 mL IC media, and at least 40 mL air into a cell inlet bag. Attach to cell line.
13. 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.
14. Take a sample of media from the EC sample port for baseline metabolite readings (QCESOM Section 5-7).
15. 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)

16. 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

17. 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

18. 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

19. Prepare a cell inlet bag with 200 mL trypsin solution and 40 mL air. Attach to Reagent line.
20. 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 Tryp LE™, 20 minutes is recommended.

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

Data

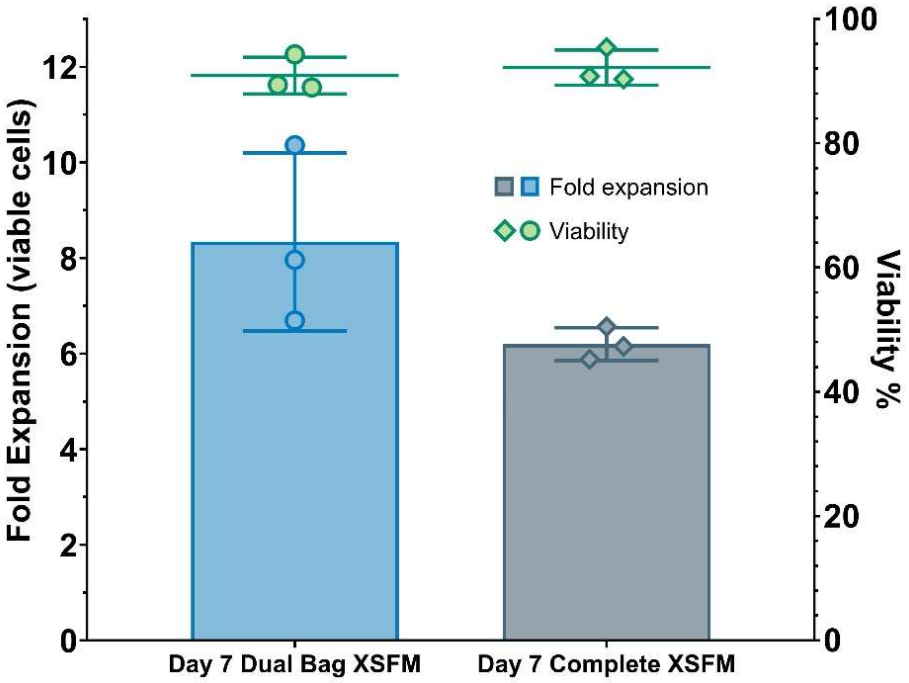


FIGURE 1. PRIME-XV MSC XSFM Dual Component Kit with Phenol Red, 91149DC performs similarly to or better than PRIME-XV MSC Expansion XSFM 91149 in 5L frozen bags. Results from three different donors demonstrate some donor-to-donor variability in fold expansion of hADMSCs. Viability is consistently maintained at or above 90% over the course of the 7-day expansion. The discrepancy in the average performance between the dual bag and complete conditions may be explained by differences in initial storage temperature. Due to the large volume (5L) of frozen media in the complete XSFM, it had to be thawed in the refrigerator for over 48 hours to completely thaw, whereas the temperature-sensitive components of the dual component bag were thawed less than an hour before the initiation of the expansion, and thus had less time to degrade.

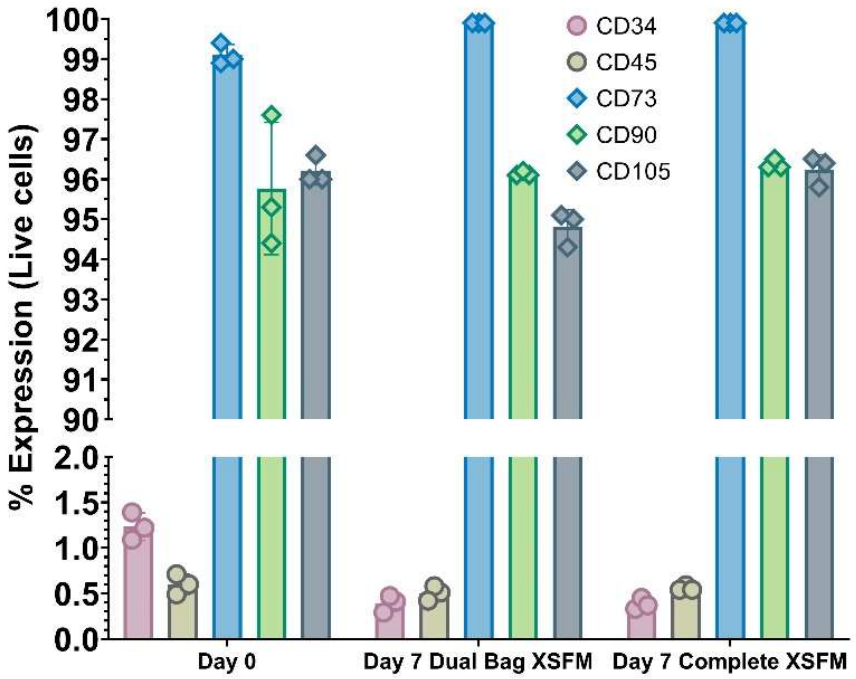


FIGURE 2. hADMSCs maintain their stem cell phenotype at day 7 post-expansion in the Terumo Quantum bioreactor and PRIME-XV MSC Expansion XSFM. The expression of negative markers CD34 and CD45 remained well below 2% over the course of the culture. The expression of the positive markers CD73, CD90, and CD105 ranged from 94-100%, indicating the maintenance of MSC differentiation potential over the course of the culture. Results are representative of three donors. All measurements were taken in triplicate.

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
91214DC	PRIME-XV MSC XSFM Dual Component Kit Without Phenol Red	Kit of 5L MSC basal and 10ml frozen supplement in bag
91149	PRIME-XV MSC Expansion XSFM	1L

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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