PRIME-XV FreezIS DMSO-Free

Catalog #	Product	Size
91140	PRIME-XV FreezIS DMSO-Free	100 mL and 10 mL liquid

Intended Use

PRIME-XV FreezIS DMSO-Free solution is recommended mainly for the cryopreservation of human mesenchymal stem/stromal cells (MSCs), $CD34^+$ hematopoietic stem cells (HSCs), T cells derived from peripheral blood mononuclear cells (PBMCs), and potentially other cell types. This product is for research use or further manufacturing use only. Not for injection or diagnostic procedure.

Product Description

PRIME-XV FreezIS DMSO-Free is a complete ready-to-use, animal component-free, and protein-free solution that does not contain dimethyl sulfoxide (DMSO). It is designed to prepare and preserve cells during frozen storage (-80°C to –196 °C), and enhance post-thaw cell viability to recover functionality.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis (CoA) which is available at www.irvinesci.com or upon request.

Shipping

This product is shipped with cold packs. Upon receipt, store immediately at 2-8°C.

Storage Instructions and Stability

Upon receipt, store PRIME-XV FreezIS DMSO-Free at 2-8°C. Unopened solution is stable for 24 months from date of manufacture. PRIME-XV FreezIS DMSO-Free should be used within 4 weeks after opening when stored at 2-8°C. Not validated for use beyond the unopened expiry shelf life.

Precautions

This product is for research or further manufacturing use only. PRIME-XV FreezIS DMSO-Free is not for use in diagnostic procedures. The safety and efficacy of this product in diagnostic or other clinical uses has not been established. Refer to the toxicity note on the PRIME-XV FreezIS DMSO-Free site for an example of non-clinical studies in animal models. This reagent should not be used beyond the expiration date. Please refer to the Safety Data Sheet for information regarding hazards and safe handling practices.

Directions for Use

The following protocol is recommended for human MSCs, PBMCs (T cells), and CD34+ HSCs. Further optimization may be required depending upon the cell type.

1. Prepare cell suspension using cell specific protocol (e.g. mechanical or enzymatic dissociation for adherent cells) and centrifuge cells as appropriate to obtain a cell pellet.

2. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet to minimize dilution of PRIME-XV FreezIS DMSO-Free.

3. Add sufficient amount of cold (2-8°C) PRIME-XV FreezIS DMSO-Free. Recommended banking density for MSCs and HSCs is 0.5-1x10^6 cells/mL, , and a minimum of 5x10^6 cells/mL for PBMCs.

4. Gently triturate cell pellet to obtain a homogeneous cell suspension.

5. Aliquot appropriate amount into cryovials.

6. Incubate cell suspension at 2-8°C for ~5 minutes.

7. Lower sample temperature to -80°C, and initiate ice nucleation (seeding) within each sample at approximately -5°C during the cooling ramp as indicated below:

    a. Use a controlled rate freezer (-1°C /minute) or similar applicable procedure for most mammalian cell types.

    b. The freezing device or isopropanol container should be pre- cooled to 2-8°C.

        i. After 15-20 minutes at -80°C, induce nucleation manually by a flick or tap of each cryovial/sample container, and return to -80°C.

    c. When using isopropanol containers, the minimum freezing time is 3 hours at -80°C.

8. Storage of frozen samples:

    a. Place samples into liquid nitrogen temperature (-196°C) for long term storage.

    b. Sample storage at -80°C* is only recommended for short-term (up to one week).

        *Human MSC & PBMC (T Cell) data available for short term storage. Human HSC data is not available.

9. Thawing procedure: Thaw frozen vial quickly in a 37°C water bath with gentle swirling of the sample until all visible ice has melted. Average thaw time for a 1mL sample in a cryovial is 2-3 minutes.

    CAUTION: DO NOT allow sample to warm above chilled temperatures (0-10°C). Cryovials should be cool to the touch when removed from the water bath.

10. Immediately dilute the mixture of thawed cells and PRIME-XV FreezIS DMSO-Free with appropriate culture medium pre-warmed to a temperature of 20-37°C at a dilution ratio of 1:10 (sample to culture media) or greater.

11. Centrifuge and remove the supernatant. This step is optional for PBMCs.

12. Resuspend cells in appropriate culture medium and transfer to culture vessel. Incubate accordingly to recover viable cells. If thawing PBMCs, rest in IL-2-containing media overnight prior to cell activation, to allow for post-thaw recovery.

Sample Data

Initial Volume	CD34+ purity	Viability pre-freeze	Viability post-thaw	Time spent frozen	Viability after 4 days in PRIME-XV Hematopoietic Cell Basal XSFM
117.0 mL	94.58%	97.7%	98.4%	93 days	96.4%
113.3 mL	97%	97.6%	93.8%	269 days	92.6%
			100%	297 days	94.8%
			100%	311 days	93.8%
			97.7%	318 days	100%
94.8 mL	98.26%	99.2%	86.4%	70 days	96.2%
			93.5%	252 days	97.4%

Table 1. Human HSCs frozen in PRIME-XV FreezIS DMSO-Free maintain a high viability post-thaw and following four days of culture in PRIME-XV Hematopoietic Cell Basal XSFM (Catalog #: 91211).

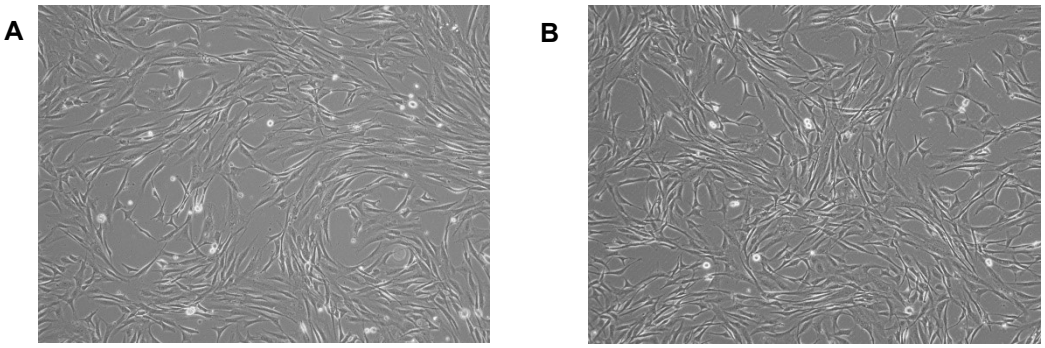

Figure 1. Attachment and morphology of MSCs cryopreserved in PRIME-XV FreezIS DMSO-Free.
Human adipose-derived MSCs were frozen at 500,000 cells/mL in PRIME-XV FreezIS (Catalog #: 91139) (A) and PRIME-XV FreezIS DMSO-Free (B). Cells were stored in liquid nitrogen for two days, then thawed and resuspended in PRIME-XV MSC Expansion SFM (Catalog #: 91135). The resuspended cells were plated at 6,000 cells/cm^2. Attachment and morphology were observed four days after thaw. Image was taken at 10X magnification.

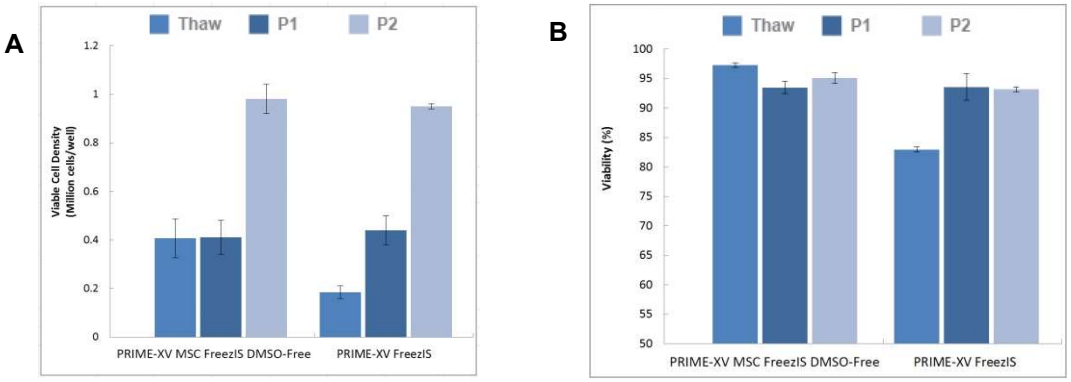

Figure 2. Human MSCs frozen in PRIME-XV FreezIS DMSO-Free had high viable cell density and percent viability. Human adipose-derived MSCs were frozen in PRIME-XV FreezIS DMSO-Free and in PRIME-XV FreezIS (Catalog #: 91139). The cells were stored in liquid nitrogen for two days before they were thawed and cultured through two passages until 80% confluent. The viable cell

density (A) and percent viability (B) were assessed with trypan blue staining in a Vi-CELL Cell Viability Analyzer at thaw and two passages post-thaw. Viable cell density was calculated using the cell count multiplied by the volume.

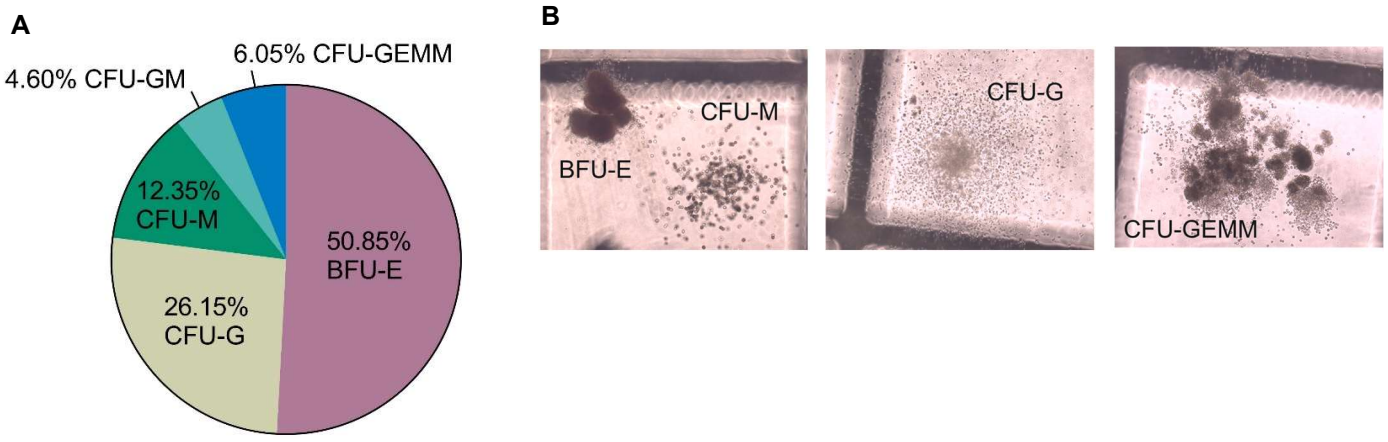

Figure 3. Human HSCs frozen in PRIME-XV FreezIS DMSO-Free maintain the ability to differentiate into a variety of myeloid cell subsets. (A) Representative proportions of differentiated myeloid cells cultured for 14 days in a semi-solid culture medium. Burst-forming unit – erythroid (BFU-E) represent approximately 50% of the population, followed by colony-forming units – granulocyte (CFU-G) and colony-forming units – monocyte (CFU-M). Mixed population colonies (CFU-GM and CFU-GEMM) account for a combined 10% of all colonies. (B) Representative images of HSC-derived colonies following 4 days of culture in 91211 PRIME-XV Hematopoietic Cell Basal XSFM (Catalog #: 91211). and 14 days of differentiation in semi-solid HSC culture media.

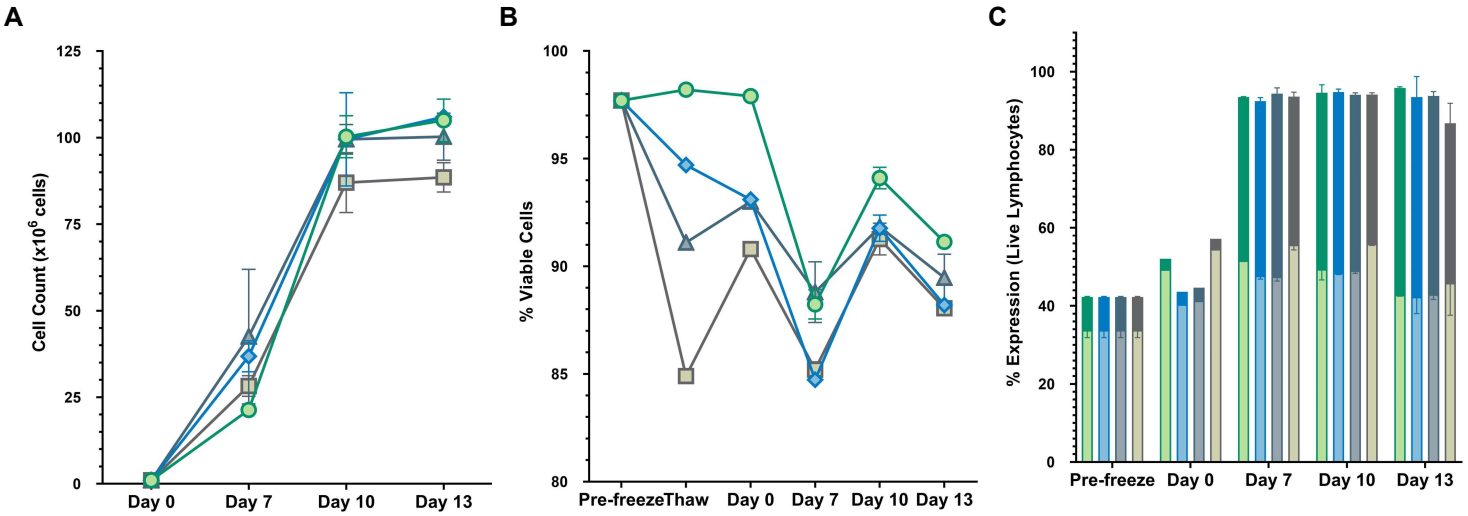

Figure 4. Human PBMCs frozen in PRIME-XV FreezIS DMSO-Free do not require a centrifugation step post-thaw (A) Cell thawed directly into culture media and cultured for 13 days yielded 100x fold expansion. Growth curve was identical to cells that were centrifuged prior to culture (data not shown). (B) PBMCs thawed from PRIME-XV FreezIS DMSO-Free directly into culture media maintained a higher viability throughout the culture, compared to those thawed from DMSO-containing media. (C) Expanded cells exhibited balanced ratio of CD4 and CD8 cells up to two weeks post-thaw.

Related Products

Catalog #	Product	Size
91135	PRIME-XV MSC Expansion SFM	1 L, 250 mL liquid
91149	PRIME-XV MSC Expansion XSFM	1 L, 250 mL liquid
91211	PRIME-XV Hematopoietic Cell Basal XSFM	500 mL liquid
91154	PRIME-XV T Cell CDM	1 L liquid
91141	PRIME-XV T Cell Expansion XSFM	1L liquid
91139	PRIME-XV FreezIS	10 mL, 100 mL liquid
500-01	CTGrade rh IL-2 $_{C126S}$	50 µg, 100 µg, 1 mg
500-07	CTGrade rh IL-7	50 µg, 100 µg, 1 mg
500-16	CTGrade rh IL-10	50 µg, 100 µg, 1 mg
500-08	CTGrade rh IL-15	50 µg, 100 µg, 1 mg
500-09	CTGrade rh IL-21	50 µg, 100 µg, 1 mg

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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