## FUJ:FILM Value from Innovation

# PRIME-XV FreezIS DMSO-Free

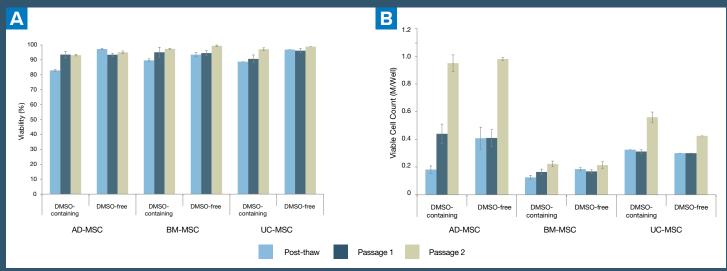
DMSO-free, protein-free, animal component-free, chemically defined cryopreservation solution

- Eliminate the risk of DMSO toxicity and maintain potency of human mesenchymal stem cells (MSCs), T cells, and hematopoietic stem cells (HSCs) throughout cryopreservation
- · Comparable post-thaw cell viability to solutions containing DMSO
- Nontoxic when injected in animal models
- Enables cell preservation for short-term storage at -80°C\* and long-term storage in liquid nitrogen to -196°C
- Complete, ready-to-use medium



\*Human MSC and PBMC (T cell) data available for short-term storage. Human HSC data is not available.

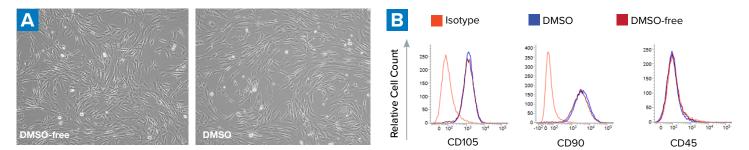




**Figure 1. PRIME-XV FreezIS DMSO-Free retains comparable MSC viable cell density and percent viability after cryopreservation compared to DMSO-containing solution.** Human adipose-derived MSCs were frozen in PRIME-XV FreezIS DMSO-Free and in PRIME-XV FreezIS. The cells were stored in liquid nitrogen for 2 days before they were thawed and cultured through 2 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 2 passages post-thaw. Viable cell density was calculated using the cell count multiplied by the volume.

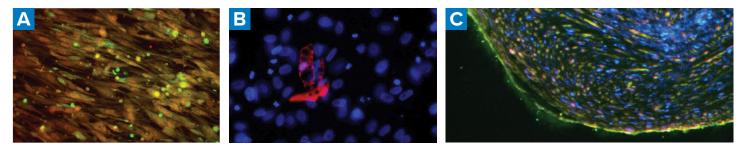


# Maintains MSC Morphology and Marker Expression



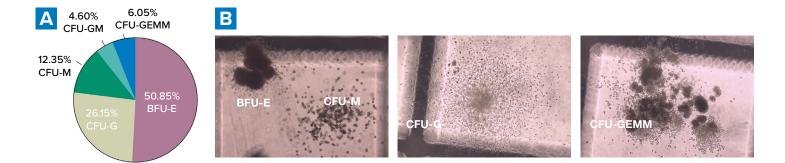
**Figure 2. PRIME-XV FreezIS DMSO-Free retains MSC morphology and marker expression after thaw and over multiple passages.** Bright field images demonstrate typical MSC morphology after thaw (A). Human AD-MSCs were cultured for 3 passages post-thaw and analyzed by flow cytometry. Expanded cells retained the characteristic MSC marker expression: positive for CD105 and CD90, and absence of CD45 (B).

## Preserves Tri-lineage Differentiation Potential



#### Figure 3. Cells cryopreserved in PRIME-XV FreezIS DMSO-Free retain differentiation potential.

Post-thawed cells were cultured for 3 passages and subsequently differentiated into osteoblasts (A), adipocytes (B), and chondrocytes (C) with PRIME-XV differentiation media. Staining for osteocalcin (green) and RUNX2 (red) shows presence of osteocytes (A). Immunocytochemistry with anti-FABP-4 antibody (red) shows differentiation into the adipogenic lineage (B). Aggrecan (red) and collagen type II (green) can be detected in a cryosectioned spheroid from day 20, demonstrating the expression of cartilaginous extracellular matrix.



**Figure 4. 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 and 14 days of differentiation in semi-solid HSC culture media.

## PRIME-XV FreezIS DMSO-Free Successfully Protects Human T Cells Potency and Viability

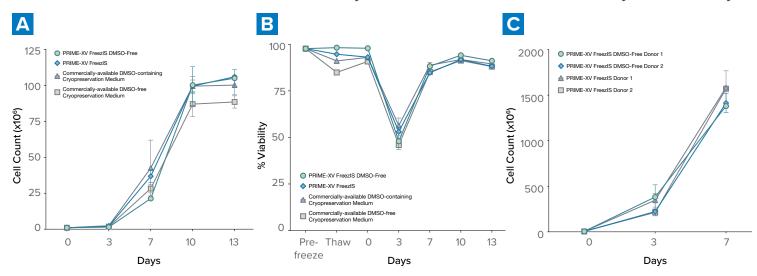
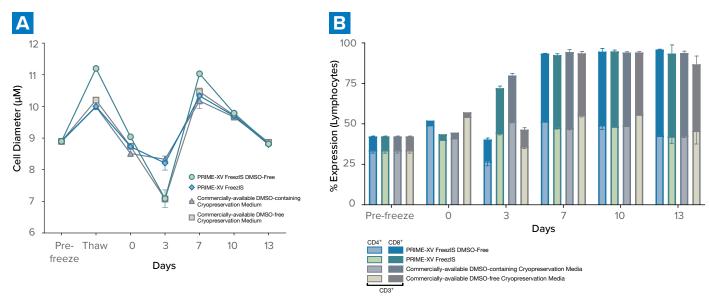


Figure 5. Cells frozen in PRIME-XV FreezIS DMSO-Free show robust expansion if plated directly into culture medium after thaw, without washing out the cryopreservation medium. Cells cultured in 24-well R-series G-Rex plates (A) and 6-well M-series G-Rex plates (C) recovered well from cryopreservation when plated directly into cell culture media post-thaw. Cells thawed from PRIME-XV FreezIS DMSO-Free media best maintained pre-freeze viability in the first 24 hours post-thaw (B). Data is representative of 3 donors.



**Figure 6. Cells thawed directly into cell culture media show similar trends in cell diameter and distribution of CD4<sup>+</sup> and CD8<sup>+</sup> cells with minor differences between DMSO-containing and DMSO-free groups. (A) Cells thawed directly into cell culture media exhibit an increase in diameter immediately post-thaw, returning to baseline after a 24-hour rest period. Cell diameter increased following cell activation using αCD3 and αCD28-conjugated beads, though was delayed slightly in DMSO-free conditions, recovering by Day 7. (B) Distribution of CD4<sup>+</sup> and CD8<sup>+</sup> cells were comparable across all conditions from day to day; however, total CD3<sup>+</sup> cell proportion among DMSO-free conditions was lower on Day 3, recovering by Day 7. Data is representative of 3 donors.** 

- FDA, Federal, and State registered cGMP-compliant manufacture
- EN ISO 13485:2016 certified
- MDSAP certified
- Extensive QC testing including functionality, sterility (USP <71>), endotoxin (USP <85>), and mycoplasma (USP <63>)
- Drug Master Files (DMFs) filed with the FDA available upon request

To discuss your requirements, contact us at getinfo@irvinesci.com or visit our website at www.irvinesci.com/contact-us.

# **Ordering Information**

Product Description	Catalog #	Size*	Additional Information
PRIME-XV FreezIS DMSO-Free	91140	10 mL 100 mL	Protein-free, chemically defined, animal component-free cryopreservation medium. Does not contain DMSO.

# **Related Products**

Product Description	Catalog #	Size*	Additional Information
PRIME-XV FreezIS	91139	10 mL 100 mL	Protein-free, chemically defined, animal component-free cryopreservation medium. Contains DMSO.
PRIME-XV MSC Expansion XSFM	91149	250 mL 1 L	Xeno-free, serum-free medium for MSC expansion
PRIME-XV MSC Expansion SFM	91135	250 mL 1 L	Serum-free medium for MSC expansion
PRIME-XV T Cell Expansion XSFM	91141	1 L	Xeno-free, serum-free T cell medium. Contains Gentamicin.
PRIME-XV T Cell CDM	91154	1 L	Chemically defined, animal component-free formula. Does not contain antibiotics or phenol red.
PRIME-XV Hematopoietic Cell Basal XSFM	91211	500 mL	Xeno-free, serum-free HSC basal medium
CTGrade rh IL-2 <sub>C126S</sub>	500-01	50 µg 100 µg 1 mg	Manufactured following cGMP practices in a facility that does not use or process beta-lactam containing materials, no histidine tags, and 0.2 micron filtered. No animal- or human-derived materials were used during manufacturing or as ingredients.
CTGrade rh IL-7	500-07	50 μg 100 μg 1 mg	Manufactured following cGMP practices in a facility that does not use or process beta-lactam containing materials, no histidine tags, and 0.2 micron filtered. No animal- or human-derived materials were used during manufacturing or as ingredients.
CTGrade rh IL-15	500-08	50 μg 100 μg 1 mg	Manufactured following cGMP practices in a facility that does not use or process beta-lactam containing materials, no histidine tags, and 0.2 micron filtered. No animal- or human-derived materials were used during manufacturing or as ingredients.
CTGrade rh IL-21	500-09	50 µg 100 µg 1 mg	Manufactured following cGMP practices in a facility that does not use or process beta-lactam containing materials, no histidine tags, and 0.2 micron filtered. No animal- or human-derived materials were used during manufacturing or as ingredients.

\*Custom sizes and packaging available upon request.





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