CryoDefend™-Stem Cells

Media for Defined, Protein-free Cryopreservation of Stem Cells

Catalog Number: CCM018

Volume: 5 x 10 mL

PRODUCT DESCRIPTION

CryoDefend-Stem Cells is a completely defined, protein-free media designed specifically for the cryopreservation of stem cells. The components have been optimized to enhance cell viability and recovery over conventional methods while maintaining potency.

INTENDED USE

The CryoDefend-Stem Cells media is designed for defined, protein-free cryopreservation of stem cells. This product contains DMSO and is ready for use.

STABILITY & STORAGE

Upon receipt, this media should be stored at ≤ -20 °C in a manual defrost freezer. The media can be thawed at 2-8 °C or at room temperature. Thawed media can be aliquoted and stored at ≤ -20 °C in a manual defrost freezer for up to 3 months. Thaw a fresh aliquot for each use. Avoid repeated freeze-thaw cycles.

PRECAUTION

When handling biohazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN 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 indicated on the label.
- Results may vary due to variations among different stem cell lines.

OTHER SUPPLIES & MATERIALS REQUIRED

- Human pluripotent stem cells
- Cryovials
- Pluripotent stem cell expansion media (*i.e.* MEF Conditioned Media, R&D Systems, Catalog # AR005)
- 15 mL centrifuge tubes
- Serological pipettes
- Pipette and pipette tips
- Centrifuge
- 37 °C and 5% CO_2 humidified incubator

PROCEDURE FOR THE CRYOPRESERVATION AND THAWING OF HUMAN PLURIPOTENT STEM CELLS

The protocol below describes the cryopreservation and thawing of pluriptotent stem cells using CryoDefend-Stem Cells media.

Note: *This protocol must be read in its entirety before using this product.*

Cryopreservation of Human Pluripotent Stem Cells

1. Thaw the CryoDefend-Stem Cells Media at 2-8 °C or room temperature.
2. Dissociate cells using dissociation solution and protocol of choice.
3. Transfer cells in suspension to a 15 mL conical tube.
4. Centrifuge the cell suspension at 200 x g for 4 minutes.
5. Remove supernatant and discard.
6. Gently resuspend cells in thawed CryoDefend-Stem Cells Media at 0.5-1.0 x 10^6 cells / mL and transfer to cryovials.
7. Freeze the cryovials under standard slow rate cooling conditions to -80 °C overnight.
8. Store the frozen cryovials in liquid nitrogen.

Thawing of Human Pluripotent Stem Cells

1. Warm pluripotent stem cell expansion media to 37 °C.
2. Carefully remove a cryovial containing frozen cells from liquid nitrogen.
3. Gently add warm expansion media to the cryovial.
4. Very gently pipette up and down and, as cells thaw, transfer the thawed portion to a 15mL conical tube containing at least 10 mL of prewarmed expansion media.
5. Centrifuge at 200 x g for 4 minutes.
6. Resuspend cells in pre-warmed expansion media and plate at the desired density.

DATA EXAMPLES

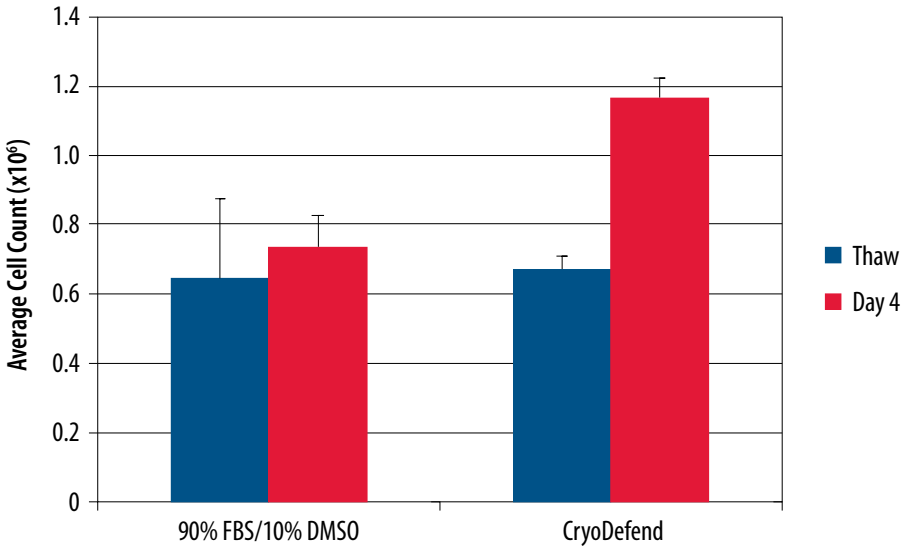

Figure 1: Human Embryonic Stem Cell Viability Following Cryopreservation in CryoDefend-Stem Cells Media and Conventional Freezing Media. BG01V human embryonic stem cells were frozen in conventional freezing media (90% FBS/10% DMSO) or CryoDefend-Stem Cells Media at 1 x 10^6 cells/cryovial and stored in liquid nitrogen for one week. The BG01V human embryonic stem cells were then thawed, counted (blue bars), and resuspended in MEF Conditioned Media (R&D Systems, Catalog # AR005) containing Recombinant Human FGF basic (R&D Systems, Catalog # 4114-TC). The resuspended cells were plated into one well of a 6-well plate and after 4 days in culture, the cells were harvested and counted (red bars). The error bars represent the standard deviation of triplicate samples.

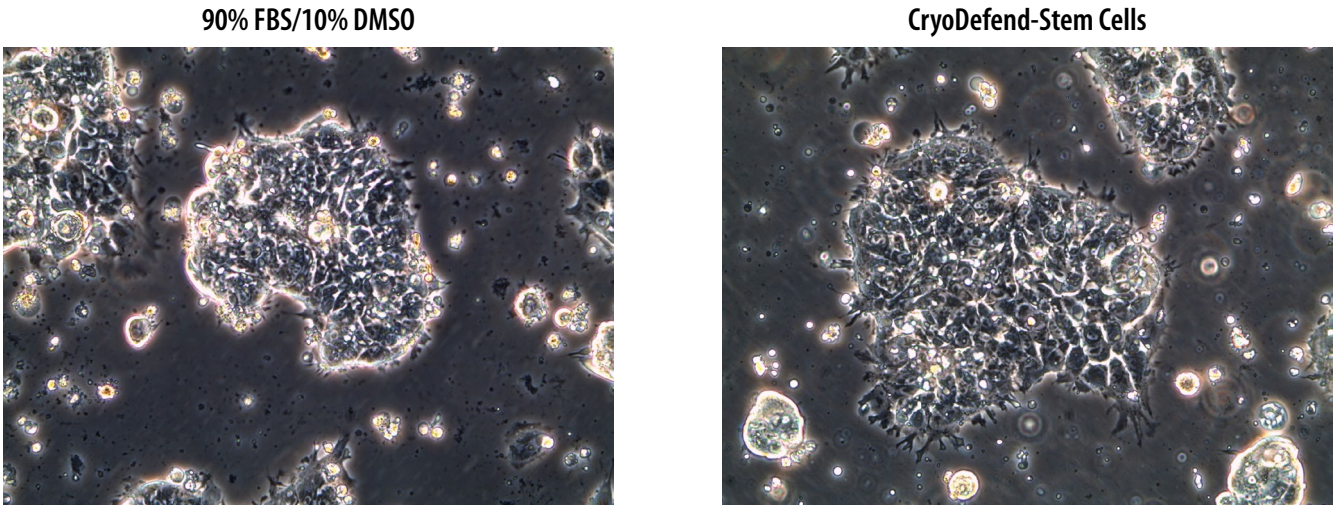

Figure 2: Morphology of Human Embryonic Stem Cells Following Cryopreservation in CryoDefend-Stem Cells Media and Conventional Freezing Media. BG01V human embryonic stem cells were frozen in either conventional freezing media (90% FBS/10% DMSO) or CryoDefend-Stem Cells media. After thawing, the cells were cultured for 3 days in MEF Conditioned Media containing Recombinant Human FGF basic and imaged using brightfield microscopy.

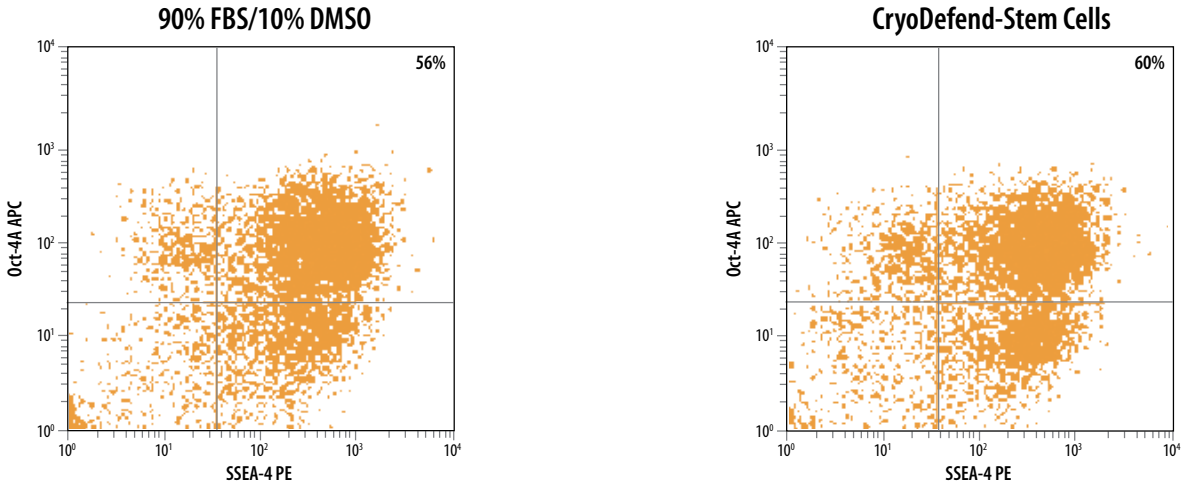

Figure 3: Expression of Pluripotency Markers in Human Embryonic Stem Cells Following Cryopreservation in CryoDefend-Stem Cells Media and Conventional Freezing Media. BG01V human embryonic stem cells were frozen in either conventional freezing media (90% FBS/10% DMSO) or CryoDefend-Stem Cells media. After thawing, the cells were cultured for 4 days and then assessed for pluripotency marker expression by flow cytometry. Cells were stained with Human Oct-4 APC Conjugated Monoclonal Antibody (R&D Systems, Catalog # IC6344A) and Human/Mouse SSEA-4 PE Conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB1435P). Quadrants were set based on isotype controls. The percent of double positive cells is indicated in the upper right quadrant.

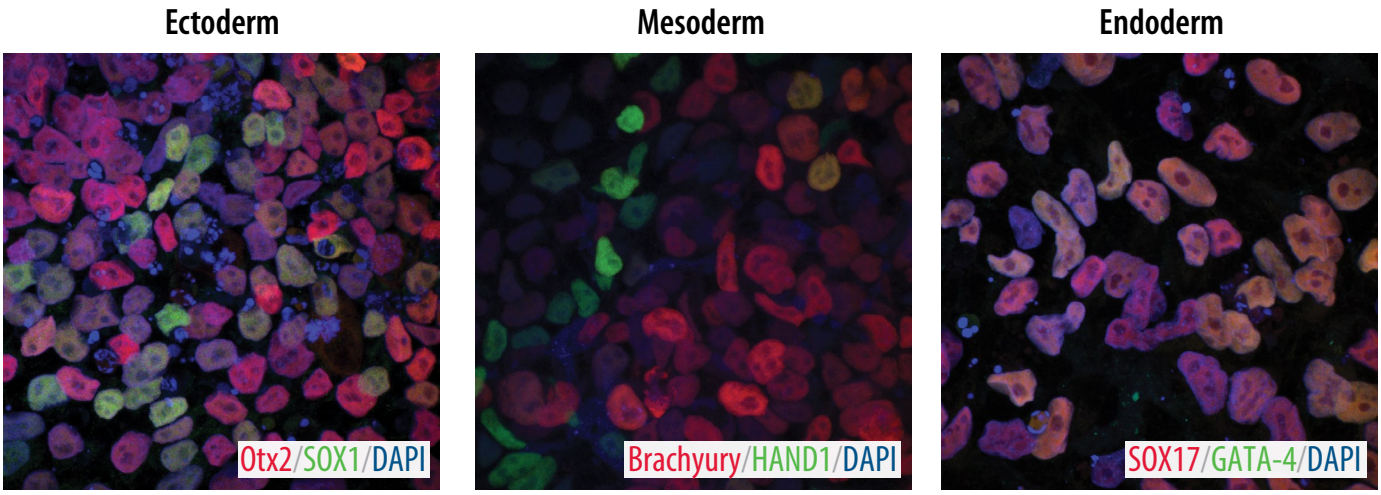

Figure 4: Human Embryonic Stem Cells Maintain Pluripotency Following Cryopreservation in CryoDefend-Stem Cells Media. BG01V human embryonic stem cells were frozen in CryoDefend-Stem Cells media. After thawing, the cells were cultured for 4 days, harvested, and replated for differentiation into each of the three germ layers according to the insert instructions for the Human Pluripotent Stem Cell Functional ID Kit (R&D Systems, Catalog # SC027). Differentiated cells were then stained for markers of each germ layer using the fluorochrome-conjugated antibodies included in the Human Three-Germ Layer 3-Color Immunocytochemistry Kit (R&D Systems, Catalog # SC022). The nuclei were counterstained with DAPI (blue).

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