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2-8CELLsius™ + DMSO (USP, Ph.Eur) Product Instructions

2-8CELLsius™ + DMSO is a cytoprotective, protein free solution, with bio-stabilizers that support high post thaw cell viability. Optimal recovery is achieved through a novel approach to cell physiology at ultra-low temperatures. Does not contain any growth factors, hormones, or antibiotics. Cells can be stored from -80°C to -196°C (liquid phase nitrogen). cGMP manufactured, USP Grade Components.

2-8CELLsius™ + DMSO is ready to use and does not require any dilution or further processing. Product is stable for a minimum of 6 months after receipt when stored at 2-8°C. Protect from light. Sterile membrane filtered.

Cell types include, but not limited to:
T Cells, iPSC, MSC, progenitor, HEK293

RECOMMENDED FREEZING AND RECOVERY PROTOCOL

FREEZING:

MIX WELL BEFORE USE. DO NOT DILUTE.

1. Examine the culture for healthy growth, confluency, etc. and the absence of contamination. Decontaminate the external surface of cell containers with 70% ethanol.
2. If freezing adherent cells, remove using 0.25% trypsin for 1 to 3 minutes at 37°C.

NOTE: Some cell lines grown in serum-free medium may be sensitive to 0.25% trypsin and therefore, may require less trypsin or the addition of a soybean trypsin inhibitor. Wash cells with 2-8CELLsius™ (Catalog# PP338) *without DMSO* after incubating with trypsin.

3. Perform a cell count to determine the total number of viable cells. Cell viability should be greater than 80%, and cells should be in late log phase or pre-confluency growth phase.
4. Centrifuge cells at 600 to 800 RPM for 10 minutes. Remove supernatant and save 3 to 5ml for sterility testing (e.g. thioglycollate, brain heart infusion, etc.) and mycoplasma testing.
5. Resuspend the cells gently in an appropriate volume of 2-8CELLsius™ + 5% or 10% DMSO at a concentration of $1x10^6$ -$1x10^7$cells/ml.
6. Dispense the cell suspension in 1 to 2ml aliquots in polypropylene cryovials or glass ampules.
7. Seal ampules and store at room temperature for 30 minutes with occasional, gentle agitation to expose cells completely to 2-8CELLsius™ + 5% or 10% DMSO.
8. In general, decrease the temperature at approximately 1°C per minute in a controlled rate freezer or in an appropriate cryo-freezing container. Store cells in vapor phase liquid nitrogen, or for long-term storage, in liquid nitrogen is acceptable.

RECOVERY:

1. Remove vials from freezer and rapidly thaw in a 37°C water bath.
2. Wipe vials with 70% ethanol.
3. Transfer cells to a 15ml centrifuge tube and slowly add 2 to 3ml of 2-8CELLsius™ (Catalog# PP338) *without DMSO* (cells are more fragile after thawing).
4. Centrifuge at 400 to 600 RPM for approximately 5 minutes.
5. Remove supernatant and transfer the cells to a culture flask with the appropriate volume of growth medium.
6. Cell viability count should be performed at least two hours after recovery.

IMPORTANT: Before terminating a culture, it is recommended that you test entire freeze/thaw cycle to ensure sterility of the culture and cell viability before long term storage.

Any questions concerning Protide products can be addressed directly to our technical support department.

FOR LABORATORY USE. NOT FOR INJECTION.

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PHARMACEUTICALS