Protide PHARMACEUTICALS

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Cellvation™ Cryopreservation Medium Product Instructions

Cellvation™ is a cryopreservative that does not contain DMSO or serum, provides optimal recovery, and is convenient to use. Cellvation™ is packaged as a 1X concentrate and should not be diluted. Cellvation™ is stable for a minimum of 6 months after receipt when stored at 4°C to 8°C.

Recommended Freezing and Thawing Protocol

Freezing:

MIX WELL BEFORE USE: DO NOT DILUTE.

1. Examine the culture for the absence of contamination, healthy growth, confluency, etc.
2. If freezing adherent cells, remove using 0.25% trypsin for 1 to 3 minutes at 37°C.
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 Cellvation™ at a concentration of $1x10^6$ - $1x10^7$ cells/ml. Some cell types such as hybridomas and myelomas may require an increase in cell density.
6. Dispense the cell suspension in 1 to 2ml aliquots in plastic or glass ampoules.
7. Seal ampoules and store at room temperature for 30 minutes with occasional, gentle agitation to expose cells completely to cryopreservative.
8. Place ampoules in an insulated container and store in a -20°C freezer for one hour. Remove insulation and transfer to -70°C freezer for one hour. (This is a critical step. The total time at -70°C must not exceed two hours.) Transfer vials to vapor phase of liquid nitrogen and store for 24 hours before transferring to liquid phase. The suggested optimum cooling rate is 1°C per minute for most cell types.

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 culture flask and **slowly** add the appropriate volume of growth medium (2 to 5ml).

4. As an alternative, transfer cells to an appropriate volume of 2-8 CELLsius™ (Protide catalog #PP338) cold storage solution. For example: 2ml for every $1x10^6$ cells. Store at 2°C to 8°C for up to 24 hours. Centrifuge and transfer to culture medium.
5. Accurate viability counts (i.e. trypan blue dye exclusion) should be performed after at least 2 hours recovery at 37°C.
6. Media should be changed once cells are settled or 24 hours after (non-adherent cells).

If desired, Cellvation™ may be removed by washing in the following manner.

1. Transfer cells to a 15ml centrifuge tube and slowly add 2 to 3ml of complete growth medium or 2-8 CELLsius™ (Protide Catalog #PP338). Cells are more fragile after thawing.
2. Centrifuge at 400 to 600 RPM for approximately 5 minutes.
3. Decant and transfer the cells to a culture flask with the appropriate volume of growth medium.
4. Cell viability count should be performed at least two hours after recovery.

IMPORTANT: Before terminating a culture, it is recommended that you test an 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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