# *Protide* Pharmaceuticals, INC.

# pZerveTM

## CRYOPRESERVATION SOLUTION

**pZerve™** is a cryopreservation solution that does not contain DMSO, fetal bovine serum or other animal protein.

Optimal recovery is achieved through a novel approach to cell physiology at ultra-low temperatures.

**pZerve™** is ready to use and does not require any dilution or further processing.

**pZerve™** is stable for a minimum of 6 months after receipt when stored at 2°C to 8°C.

MIX WELL BEFORE USE. DO NOT DILUTE. **pZerve™** is sterile membrane filtered.

Catalog number 5700 Size 60ml Catalog number 5720 Size 20ml

**RECOMMENDED FREEZING AND THAWING PROTOCOL**

**FREEZING:**

1. Examine the culture for healthy growth, confluency, etc. and the absence of contamination.

2. If freezing adherent cells, remove using 0.25% trypsin for 1to 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 trypsin inhibitor. Wash cells 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 5 ml for sterility testing (e.g. thioglycollate, brain heart infusion, etc.) and mycoplasma testing.

5. Resuspend the cells gently in an appropriate volume of **pZerve™** 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 1to 2ml aliquots in plastic or glass ampules.

7. Seal ampules and store at room temperature for 30 minutes with occasional, gentle agitation to expose cells completely to cryopreservative.

8. Place ampules 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. Do not store at -70°C for more than 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 5 ml).

4. As an alternative, transfer cells to an appropriate volume of 2-8 CELLsiustm (Protide catalog #PP338) cold storage solution. For example: 2ml for every x106 cells. Store at 2-8 ° 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. Medium should be changed within 24 hours.

If desired, **pZerve™** may be removed by washing in the following manner:

1 Transfer cells to a 15ml centrifuge tube and slowly add 2 to 3 ml of complete growth medium (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 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.

**pZerve™** only contains human protein.

CAUTION: The venous blood from which this product is manufactured has been tested for the

presence of Hepatitis B Surface Antigen (HBsAg) and found to be non-reactive by a currently

approved FDA test. The blood has also been tested for the presence of HIV antibody and found to be

negative. However, in accordance with Good Manufacturing and Laboratory Practices, any protein of human

blood origin should be handled as if capable of transmitting hepatitis or other infectious agents.

For Laboratory Use. Not For Injection.

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