

## Recommended Freezing and Thawing Protocol

MIX WELL BEFORE USE. DO NOT DILUTE.

### Freezing:

- 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 5 mL for sterility testing (e.g. thioglycolate, brain heart infusion, etc.) and mycoplasma testing.
- 5 Resuspend the cells gently in an appropriate volume of Cell Cryopreservation Medium with 10% DMSO at a concentration of  $1 \times 10^6$  -  $1 \times 10^7$  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 2 mL 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. (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 5 mL).
- 4 As an alternative, transfer cells to an appropriate volume of 2-8 CELLsium™ Medium (Cat. No. 0927803 from MP Bio) short-term cold storage solution. For example: 2 mL for every  $1 \times 10^6$  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, the cryopreservation medium may be removed by washing in the following manner:

- 1 Transfer cells to a 15 mL centrifuge tube and slowly add 2 to 3 mL of complete growth medium or 2-8 CELLsium™ Medium (Cat. No. 0927803 from MP Bio). 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 2 hours after recover.

**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.

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