PROTOCOL

Cell Cryopreservation Medium with 10% DMSO

Cat. No. 092780248

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 $1x10^6$ - $1x10^7$ 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 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 $1x10^6$ 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, 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.

MP Biomedicals
Americas: 800.854.0530 | custserv@mpbio.com
Europe: 00800.7777.9999 | custserv.eur@mpbio.com
APAC: 65.6775.0008 | enquiry_ap@mpbio.com