HyClone™ media and supplements

HyCryo 2× cryopreservation medium for general use

FOR RESEARCH USE ONLY

Overview

HyCryo cryopreservation medium is designed for general use and is suitable for freezing down many different cell types including CHO-K1, VERO, MDCK, and HEK293 cells. This formulation contains DMSO and its components are animal-derived component-free. HyCryo medium is provided at a 2× concentration to be added to cells resuspended in their own conditioned or fresh growth media.

Required materials

- 100 mL HyCryo 2× cryopreservation medium SR30001.02 (or 50 mL made to order, SR30001.01)
- Cell harvesting solution (as appropriate for cell type to be used)
- Conditioned or fresh growth medium (as appropriate for cell type to be used)
- Cryopreservation vials
- Shelf-freezing container with isopropanol

Storage, handling, and stability

Upon receipt, store cryopreservation medium at -10°C or lower. Medium is stable at -10°C or lower for up to 24 months and up to 6 months at +4°C once thawed. Medium may be refrozen for storage at -10°C or lower. Store in aliquots to avoid repeated freeze/thaw cycles.

Protocol

Freezing cells

1. Save a portion of the conditioned growth medium from the cell culture. If desired, centrifuge or filter to remove any dead floating cells that may be present.
2. Harvest cells according to method recommended for your desired cell type.
3. Determine cell number using a hemocytometer or other cell counting method.
4. Centrifuge desired number of cells to pellet. Aspirate supernatant.
5. Resuspend cells in chilled conditioned medium to a cell density of ~ 2 × 10^6 cells/mL.
6. Gently with swirling, add an equal volume of chilled 2× cryopreservation medium.
7. Aliquot cell suspension to cryopreservation vial (1mL/vial). Keep cell suspensions chilled.
8. Transfer vials to chilled isopropanol shelf-freezing container and place in a -80°C freezer for 6 to 72 h to slow-freeze the cells.
9. Transfer vials to liquid nitrogen container for long term storage.

Initiating cell culture

1. Quickly thaw cells in 37°C water bath. Remove from the water bath before the ice has completely melted.
2. Spray the vial with 70% ethanol and transfer to biosafety cabinet.
3. Transfer 1 mL cell mixture to ~ 10 mL warm growth medium, drop-wise with swirling. Use warm growth medium to thaw any remaining ice in vial. Gently mix cell suspension.
4. Centrifuge cells to pellet. Aspirate supernatant.
5. Resuspend cells in warm growth medium and plate as recommended for your cells.

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