

# HyCryo-STEM cryopreservation medium

## HYCLONE MEDIA AND SUPPLEMENTS

HyClone™ HyCryo-STEM medium is a serum-free product intended for cryopreservation and storage in biomanufacturing of stem cell lines. The medium is designed for use with neural progenitor or stem cells, embryonic stem cells (ESCs), and induced pluripotent stem (iPS) cells. This serum-free formulation contains DMSO and protein components to protect cells during the cryopreservation process. HyCryo-STEM medium is provided at a 2× concentration to be added to cells resuspended in their own conditioned or fresh growth medium (Fig 1).

### Key benefits of HyCryo-STEM

- Designed to freeze cells traditionally sensitive to the cryopreservation process
- Serum-free to ensure lot-to-lot consistency
- Provided at a 2× concentration for addition to cells suspended in their own conditioned growth medium to minimize osmotic shock during cryopreservation
- Helps maintain cell stemness, providing healthy and stable stocks of stem cells for downstream applications

## Product storage and handling

Upon receipt, store HyCryo-STEM cryopreservation medium at -20°C. The medium is stable at -20°C for up to 24 months and up to 6 months at 4°C after thawed. Medium may be refrozen for storage at -10°C or lower. Store in aliquots to avoid repeated freeze/thaw cycles.

## Instructions for use

### 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 might 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  $\sim 2 \times 10^6$  cells/mL.
6. Gently with swirling, add an equal volume of chilled 2× cryopreservation medium.
7. Aliquot cell suspension to cryopreservation vial (1 mL/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 hours to slow-freeze the cells.
9. Transfer vials to liquid nitrogen container for long-term storage.



Fig 1. HyCryo-STEM cryopreservation medium.

## 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.

## Ordering information

Product	Size	Product code
HyClone HyCryo-STEM	100 mL	SR30002.02

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