

# COOL CELLS, HOT RESEARCH



Keep your cells protected and your experiments on track with our special cryopreservation bundle.

- Cell Freezing Reagents
- Cryogenic Vials
- Storage Racks
- Protective Gloves

With everything you need in one package, saving your cells becomes seamless so you can focus on what really matters: making great discoveries!

Don't miss out on this limited-time deal! Use promo code **IIH** at checkout.

Description	Product code	Discount
<b>Dimethyl sulfoxide</b>		
Hybri-Max™, sterile-filtered, BioReagent, suitable for hybridoma, ≥99.7%	<a href="#">D2650</a>	40%
Hybri-Max™, 0.2 µm filtered, BioReagent, suitable for hybridoma, ≥99.7%, Ampoule	<a href="#">D2653</a>	40%
≥99.5% (GC), suitable for plant cell culture	<a href="#">D4540</a>	40%
sterile-filtered, BioPerformance Certified, meets EP, USP testing specifications, suitable for hybridoma	<a href="#">D2438</a>	40%
meets EP testing specifications, meets USP testing specifications	<a href="#">D1435</a>	40%
<b>Cell Freezing/Cryopreservation Media</b>		
Cell Freezing Medium-DMSO 1x	<a href="#">C6164</a>	20%
	<a href="#">C6295</a>	20%
CryoSOfree™ DMSO-free Cryopreservation Medium	<a href="#">C9249</a>	20%
CryoStor® cell cryopreservation media	<a href="#">C2874</a>	20%
CryoStor® cell cryopreservation media	<a href="#">C2999</a>	20%
HypoThermosol® FRS Preservation Solution	<a href="#">H4416</a>	20%
<b>NEW</b> Cryo-DMSO-F cryopreservation medium serum-free, protein-free, animal origin-free, and DMSO-free"	<a href="#">C9252</a>	25%

**NEW**

**CRYO-DMSO-F** is a fully defined, DMSO-free cryopreservation medium for ultra-low temperatures (-80°C to -196°C). It's serum-free, protein-free, and animal origin-free—ideal for sensitive cells like CAR-T, NK, and stem cells. Key benefits include quick, ready-to-use formulation, improved functionality without DMSO, and sterile filtration to reduce contamination risks



Description	Product code	Discount
<b>Cryogenic Vials and Storage Racks</b>		
MTC™ Bio Sterile Cryogenic Vials, capacity 5 mL, thread style external	<a href="#">MTCV3805-500EA</a>	30%
MTC™ Bio Sterile Cryogenic Vials, capacity 2 mL, thread style internal	<a href="#">MTCV4802-500EA</a>	30%
MTC™ Bio Locking Rack for Cryogenic Vials, to hold, 5 x 10 cryovials	<a href="#">MTCV5920-4EA</a>	30%
MTC™ Bio Cap Inserts for Cryogenic Vials, green	<a href="#">MTCV5809G-500EA</a>	30%
MTC™ Bio Cap Inserts for Cryogenic Vials, assorted colors	<a href="#">MTCV5809A-500EA</a>	30%
MTC™ Bio Cap Inserts for Cryogenic Vials, white	<a href="#">MTCV5809W-500EA</a>	30%
Nalgene® cryogenic vials, capacity 2.0 mL, sterile, External threads	<a href="#">V5007-500EA</a>	30%
Nalgene® cryogenic vials, capacity 2.0 mL, sterile, External threads, pkg of (Bulk packed)	<a href="#">V5132-1000EA</a>	30%
Corning® cryogenic vials, internal thread, capacity 2.0 mL, bottom, round, seal, washer, self-standing, case of 500	<a href="#">CLS430488-500EA</a>	25%
BRAND® cryogenic tube with screw cap, capacity 2 mL, Internal thread, self-standing	<a href="#">BR114841-1000EA</a>	15%
Nalgene® CryoBox™, 9 x 9 Array For 1.2 and 2.0 mL vials	<a href="#">R0888-4EA</a>	30%
Nalgene® CryoBox™, CryoBox 100, 10 x 10 array	<a href="#">Z359017-10EA</a>	30%
<b>Protective Gloves</b>		
Cryo-gloves®, size L, water-resistant	<a href="#">Z183520-1PAK</a>	10%
Cryo-gloves®, size M, water-resistant	<a href="#">Z183512-1PAK</a>	10%
Cryo-gloves®, size L, waterproof	<a href="#">Z183563-1PAK</a>	10%
<b>Cryo Labels</b>		
Cryo-Babies® Labels, white, L x W 1.28 in. x 0.5 in., roll of 1000 labels	<a href="#">Z366218-1PAK</a>	30%
Laser Cryo-Tags®, white, L x W 1.5 in. x 0.75 in.	<a href="#">Z688584-1PAK</a>	30%
Laser Cryo-Babies®, L x W 15/16 x 1/2, white	<a href="#">Z742254-1PAK</a>	30%



Visit our Cell Freezing Reagents webpage to discover our comprehensive offer; apply promo code **IIH** and save up to 40%.

**Offer expires on 31 December 2025**

## Cell Freezing Tips and Tricks\*

\*ECACC Laboratory Handbook 4<sup>th</sup> Edition

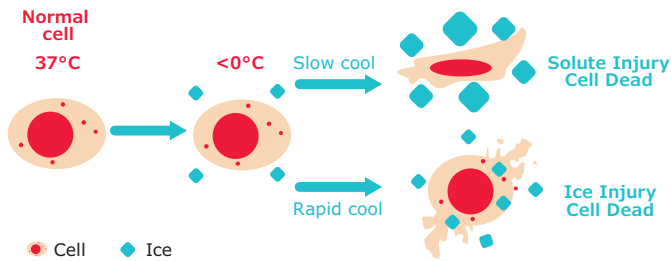
Cryopreservation of cell lines preserves valuable laboratory resources. Improper freezing can damage cells, disrupt experiments, and negatively affect data collection.

### Cryopreservation of Cell Lines

Significant developmental work has been done to ensure successful cryopreservation and resuscitation of various cell lines. The key principle is a slow freeze and quick thaw. Generally, cells should cool at -1 °C to -3 °C per minute and thaw quickly in a 37 °C water bath for 3-5 minutes.

Following these guidelines and the points below will help achieve successful cryopreservation for most cell lines.

1. Cultures must be healthy with >90% viability and no microbial contamination.
2. Use cultures in the log phase of growth, achieved by using pre-confluent cultures and changing the medium 24 hours before freezing.
3. Use a high concentration of serum/protein (>20%), often 90%.
4. Employ a cryoprotectant like dimethyl sulphoxide (DMSO) or glycerol to protect cells from ice crystal rupture. DMSO at 10% is common, but glycerol is an alternative when DMSO induces differentiation (refer to ECACC data for the correct cryoprotectant). Ready-made cell freezing media with DMSO, glycerol, and serum-free options are available.
5. Slow freezing at approximately 1 °C per minute using a Nalgene Mr. Frosty or Corning Cool Cell Freezing Container can enhance cryopreservation success.



**Cell cryopreservation mechanism.** Cryoprotectants, such as DMSO or glycerol, may be added to cell culture media as a cryoprotectant for cells. DMSO reduces ice crystal formation and thereby prevents cell death during the freezing process. Approximately 10% v/v may be used with a slow-freeze method (decreasing the temperature approximately 1°C per minute) and the cells may be frozen at  $-80^{\circ}\text{C}$  ( $-112^{\circ}\text{F}$ ) or stored in liquid nitrogen for extended periods of time.

## Tiered Cell Banking System

Upon acquiring a new cell line, a small set of 3–5 ampoules is frozen as a “token” stock before creating a master cell bank (MCB). One ampoule is tested for mycoplasma; if it fails, the process stops. If successful, another ampoule is resuscitated to produce an MCB of 10–20 ampoules, verified through quality checks like viability, cell count, mycoplasma tests, and cell line authentication (e.g., STR profiling). If any tests fail, the banking procedure must be repeated.

Once the MCB is acceptable, a working cell bank (WCB) is prepared by expanding cells from one MCB ampoule, typically ranging from 20 to 200 ampoules. Similar quality checks are performed. Before using any WCB

ampoule, tests must confirm cell viability, absence of contaminants, and correct identity. Cells should only remain in culture for a limited number of doublings to prevent genetic drift; once this limit is reached, the WCB is replenished from new MCB ampoules.

It’s crucial to maintain enough master cell ampoules for future WCB generation. If stocks fall below five ampoules, a fresh MCB can be constructed. Regulatory authorities may require additional tests like viral screening or karyotype checks. Consistent documentation of bank size, freezing dates, passage numbers, and QC results is vital for reproducible research and production.

## Thawing of Frozen Cell Lines

1. Read the cell line data sheet for specific requirements.
2. Label flasks with the cell line name, passage number, and date.
3. Collect an ampoule from liquid nitrogen, handling with care to avoid explosion.
4. In a safety cabinet, disinfect the ampoule cap with 70% alcohol, then thaw in a  $37^{\circ}\text{C}$  water bath for 1-2 minutes.
5. NOTE: Do not immerse the ampoule completely to reduce contamination risk.
6. Wipe the ampoule with 70% alcohol before opening.
7. Transfer the entire ampoule content to a sterile tube, adding 5 mL pre-warmed medium. Determine viable cell density with trypan blue and adjust for seeding density.
8. For adherent cells, adjust medium volume to recommended seeding density; a pre-centrifugation step is usually unnecessary. For immediate use, consider pre-centrifugation.
9. For suspension cells, centrifuge at  $150 \times g$  for 5 minutes to remove cryoprotectant, then resuspend in fresh medium.
10. Incubate at the recommended temperature and  $\text{CO}_2$  level; use vented caps for gaseous exchange.
11. Examine cells microscopically after 24 hours and sub-culture as needed.

