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Technical Datasheet

# CryoXL™ Hybridoma Cell Freezing Medium, DMSO, 1X

**With FBS and DMSO**
**Without Antibiotics**
**Sterile filtered**
**This medium is optimized for cryopreservation of hybridoma cells**

**Product Code: TCL123**

## Product Description:

HiMedia's Cell Freezing Media are complete, ready to use reagents designed to protect and preserve cells during frozen storage. These media are a convenient and cost effective alternative to in-house freezing media and can be used for a wide variety of mammalian cells.

TCL123 is Hybridoma Cell Freezing Medium formulated to contain 10% DMSO and fetal bovine serum. The medium does not contain antibiotics. DMSO acts as a cryoprotectant and prevents formation of ice crystals and prevents cell damage. It is ready to use and does not require further addition of any other reagents.

This medium is optimized for cryopreservation of sensitive hybridoma cell lines. Users are advised to test the suitability of the medium for individual cell lines.

## Composition:

This medium is a proprietary formulation.

## Directions:

Cell freezing medium can be used with standard freezing protocols. The following protocol may be used.
Thaw cell culture freezing medium, mix well, and keep on wet ice during use.

**Procedure for freezing:**

1. For optimum results, cells should be in log phase of growth.
2. Gently pellet the cell suspension by centrifugation (200 to 400 × g for 5 minutes). Using a pipette, remove the medium above the pellet down to the smallest volume without disturbing the cells.
3. Resuspend the cells in Cell Freezing Medium at a recommended density for a specific cell type. Hybridoma cells require higher cell density (5 to 10 × $10^6$ cells/ml).
4. Aliquot cells in appropriate cryogenic storage vials. Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately 1ºC per minute. Alternatively, place the cryovials containing the cells in an isopropanol chamber and store them at -80ºC overnight. Alternatively, store them at -20ºC for 1 - 2 hours before shifting to -80ºC overnight.
5. Transfer cryovials to vapour phase of liquid nitrogen tank for long term storage.

**Procedure for thawing of cryopreserved cells:**

1. Remove cells from frozen storage and quickly thaw in a 37ºC water bath.
2. Dilute 1ml suspension with 10ml of complete growth medium.
3. Mix cells gently and pellet by gentle centrifugation.
4. Discard the supernatant and gently resuspend the cells in complete growth medium and seed in appropriate culture vessel.
5. It is recommended to do viability assessment 24 hours post thawing.

**Notes:**

1. Cells harvested for cryopreservation should be at their optimum viability to ensure maximum survival during freezing and after thawing.
2. On removal from storage, extreme caution must be exercised to prevent explosion of the cryovial because of sudden expansion of the trapped nitrogen.
3. To retain maximum viability during cryopreservation, cells must be cooled at a constant slow rate, -1 to -5ºC/min. This can be achieved using programmable freezers or placing ampoules in a heavily insulated box at -80ºC for 24 hours before transferring them to their final storage location.
4. After thawing cells, it is necessary to slowly dilute the croprotectant to prevent osmotic shock. When it is necessary to centrifuge the cells, use the minimum g force to sediment them to prevent shearing damage, i.e. 70-100g.
5. To initiate rapid growth, it is advisable to inoculate new cultures at a higher density than for routine subculture, e.g., 2 to $5 \times 10^5$ viable cells/ml.
6. Sometimes hybridoma cultures take many days to recover from cryopreservation. Most of the time, viability of cells declines on day one and then the cells begin to recover and enter into the exponential phase.
7. The minimum number of tests that should be carried out on master cell banks are, total and viable cell counts, growth potential, screening for bacteria, fungi and mycoplasma and cell line authenticity.

## Quality Control:

**Appearance**
Amber colored clear solution.

**pH**
7.20 - 7.80

**Sterility**
No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

**Performance Test**
Performance test is done by freezing cells and doing a viability assessment after thawing and comparing it with a control medium.

**Post-thaw viability of AE-1**
85%

**Post-thaw viability of DC101**
85%

## Storage and Shelf Life:

Cell Freezing Media should be stored at -30°C to -10°C. For frequent use, cell freezing medium once thawed can be stored at 2-8°C up to 5 days.
The shelf life of product is 12 months.
Use before expiry date given on the product label.