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Technical Datasheet

# CryoXL™ Cell Freezing Medium-DMSO, 1X

**With FBS and DMSO**
**Without Antibiotics**
**Sterile filtered**

**Product Code: TCL043**

## 1. Intended use:

For cryopreservation of hardy cell lines such that are less susceptible to freezing damage. For Research Use Only. Not for use in diagnostic procedures.

## 2. Product description:

CryoXL™ 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.

TCL043 is a DMSO containing cell freezing medium, prepared in Dulbecco's Modified Eagle Medium. It contains 10% DMSO and fetal bovine serum. DMSO acts as a cryoprotectant and prevents formation of ice crystals and prevents cell damage. FBS protects the cells from detrimental effects of DMSO, improves the post-freezing recovery and survival after cryopreservation. This is a ready to use medium and does not require further addition of any other reagents.

## 3. Composition:

| Ingredients | %V/V |
|---|---|
| **OTHERS** | |
| Dimethylsulphoxide (DMSO) | 10% |
| Dulbecco's Modified Eagle Medium | Made to volume |
| Fetal bovine serum | Proprietary |

## 4. Cryopreservation procedure:

### 4.1. Cryopreservation pre-requisites

4.1.1. Cells should be healthy and should be in log- phase of the growth.

4.1.2. The freezing medium should be kept on ice all the time during cryopreservation process.

4.1.3. The cryovials should be pre-labelled with necessary information.

4.1.4. If using isopropanol chamber for controlled freezing, the chamber should be pre-chilled at -80ºC overnight.

4.1.5. The liquid nitrogen tank should contain acceptable volume of the liquid nitrogen filled into it.

### 4.2. Materials required but not provided

Dissociation reagent
Cryovials
Centrifuge tubes
Serological pipettes
Pipette aid and tips
Centrifuge machine
Isopropanol chamber / deep freezer (-30ºC to -10ºC) / deep freezer (-80ºC)
Liquid nitrogen tank with acceptable level of liquid nitrogen

### 4.3. Cryopreservation

**THE PROCESS OF CRYOPRESERVATION SHOULD BE SLOW**

4.3.1. Dissociate the adherent cells from the vessel surface using suitable dissociation reagent.

4.3.2. Gently pellet the cell suspension by centrifugation (200 to 400g for 5 minutes for suspension cells and 200g for 5 minutes for adherent cells).

4.3.3. Using a pipette, carefully remove the medium above the pellet down to the smallest volume without disturbing the cells.

4.3.4. Resuspend the cells in the chilled cell freezing medium at a recommended density, specific cell line being frozen. Hybridoma cells may require higher cell density.

4.3.5. Aliquot the cells in appropriate pre-labelled cryovials. There are three options for slow-freezing of the cells –

Option 1 - Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately -1ºC per minute.

Option 2 - Place the cryovials containing the cells in a pre-cooled isopropanol chamber and store them at -80ºC overnight.

Option 3 - Store the vials at -20ºC for 1 - 2 hours before shifting to -80ºC overnight.
3.6. Transfer the cryovials to the vapour phase of liquid nitrogen tank for long term storage.
Note: Do not immerse the vials in liquid phase of the tank. Make sure that the vials are stored in vapour phase.

## 5. Revival procedure:

### 5.1. Revival pre-requisites

5.1.1. The complete growth medium should be equilibrated at 37ºC, 5% $CO_2$ for minimum 2 hours prior to adding thawed cells.

5.1.2. The culture vessels should be pre-labelled with necessary information.

5.1.3. Water bath should be filled with clean water & equilibrated at 37ºC.

### 5.2. Recommendations for revival

5.2.1. To initiate rapid growth, it is advisable to inoculate new cultures at a higher density than for routine subculture, e.g., between 3 and 4 X 104 viable cells/cm2 for adherent cells

5.2.2. 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.

### 5.3. Materials required but not provided

Complete growth medium
Appropriate culture vessel
50ml centrifuge tube
Trypan blue solution (TCL005)
Serological pipettes
Pipette aid and tips
$CO_2$ Incubator
PPE for handling liquid nitrogen tank and cryopreserved vials

### 5.4. Revival

**THE PROCESS OF REVIVAL SHOULD BE VERY QUICK**

5.4.1. Wear appropriate PPE before handling the liquid nitrogen tank.

5.4.2. Remove the cryovial from the cryo-storage.

5.4.3. Quickly thaw the vial partially in a 37ºC water bath (in less than 2 minutes) and bring it in the biosafety cabinet.

5.4.4. Disinfect the vial surface by spraying 70% isopropanol. There are two options for further processing of the cells

**Option 1 – Direct revival in the culture vessel (Recommended for adherent cells)**

5.4.5. Add the cell suspension drop by drop to the flask containing the 10ml pre-warmed complete medium. Keep swirling the flask while adding the cell suspension.

5.4.6. Cap the flask and shake gently to ensure proper mixing and uniform distribution of cells in the medium.

5.4.7. Take out an aliquot of the revived cell suspension and determine cell density and viability by Trypan blue dye exclusion method.
5.4.8. Incubate the flask for 2-3 hours at 37ºC, 5% $CO_2$. If more than 70-80% cells are attached, replace the medium with fresh medium and incubate further.

**Option 2 – Centrifugation prior to addition in vessel (Recommended for suspension cells)**

5.4.1. Add the cell suspension drop by drop to the 50ml centrifuge tube containing the 10ml pre-warmed complete medium. Keep swirling the tube while adding the cell suspension.

5.4.2. Pellet the cells by gentle centrifugation at 70-100g.

5.4.3. Discard the supernatant and resuspend the cells in complete growth medium. Seed the suspension in suitable culture vessel.

5.4.4. Take out an aliquot of the revived cell suspension and determine cell density and viability by Trypan blue dye exclusion method.

5.4.5. Incubate the flask at 37ºC, 5% $CO_2$.

## 6. Quality Control:

**Appearance**
Red colored clear solution.

**pH**
7.60 - 8.20

**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.

## 7. 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.