FREEZin1™

With DMSO
Without Antibiotics, Antimycotics and Phenol red
Sterile filtered

Product Description :

Product Code: TCL098

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. TCL098 is chemically defined serum free cell freezing medium designed to freeze the cells grown in serum free and animal component free conditions. This medium ensures high cell viability upon revival from cryo-storage and can also be used for cryopreservation of stem cells and primary cells.
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. For adherent cells: Gently detach the cells from the surface using Trypsin or other non-enzymatic dissociation agents. Cells grown in serum free conditions may require less than 0.25% trypsin and the addition of trypsin inhibitor.
3. For suspension cells: Directly aliquot the cell suspension in sterile centrifuge tube. Gently pellet the cell by centrifugation (200 to 400 x g for 5 minutes for suspension cells and 200 x g for 5 minutes for adherent cells). Using a pipette, remove the medium above the pellet down to the smallest volume without disturbing the cells.
4. Resuspend the cells in Serum Free Cell Freezing Medium at a recommended density for a specific cell type.
5. 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.
6. Transfer cryovials to 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 assess viability 24 hours post thawing. For accurate assessment, it is recommended to use fluorescent assay like EZBlue™ (CCK004 - EZBlue™ Cell Assay Kit) or metabolic assays like MTT (CCK003 - EZcount™ MTT Cell Assay Kit).
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., between 3 and 4 x 104 viable cells/cm2 for adherent cells.
6. 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
Colorless, clear solution.

pH
7.60 -8.20

Osmolality in mOsm/Kg H_2O
1800.00 -2200.00

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.

Storage and Shelf Life:

Upon receipt, store the product at -20°C in a freezer that is not self-defrosting. Once thawed, the product is table for up to 5 days at 2 - 8°C.
Repeated freezing and thawing is should be avoided. Once thawed, the product can be aliquoted in smaller volumes and frozen for future use.
The shelf life of product is 12 months.
Use before expiry date given on the product label.

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