CryoXL™ Stem Cell Freezing Medium

With FBS and DMSO
Without Antibiotics
Sterile filtered

Product Code: TCL107

Product Description :

HiMedia's 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.

TCL107, CryoXL™ Stem Cell Freezing Medium is a fully supplemented proprietary formulation designed for cryopreservation of human stem cells. It contains fetal bovine serum and DMSO. 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 supplements.

Human stem cells cryopreserved using this medium exhibit optimal cell viability and recovery upon revival while maintaining their multipotency and morphology. Users are advised to test the suitability of the medium for different types of human stem cells.

Directions :

Thaw cell freezing medium, mix well by gentle swirling, and keep on wet ice during use.

Procedure for freezing:
1. For optimum results, cells should be in log phase of growth.
2. Gently detach adherent cells from the surface using Trypsin or other appropriate means.
3. Gently pellet the cell suspension by centrifugation at 200g for 10 minutes.
4. Resuspend the cells in cold Cell Freezing Medium at a recommended density for a specific cell type.
5. Aliquot the cells in appropriate cryogenic storage vials and start the freeze down procedure immediately. 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. Aseptically add 10ml of pre-warmed complete medium to the flask.
2. Place frozen vial of cells in the water bath with lower half immersed in water. Keep shaking the vial until the frozen clump inside it is 70% thawed.
Note: Do not vortex the cells. Avoid getting the water up to the cap of the vial to decrease the chance of contamination.
3. Swab the vial thoroughly with 70% isopropanol and open it in a laminar hood.
4. Immediately transfer the contents of the vial to the flask containing complete medium drop by drop with intermittent shaking of the flask.
5. Rock the flask gently to ensure proper mixing of cell suspension and medium.
6. Incubate the flask in horizontal position in an incubator at 37ºC and 5% CO_2 atmosphere.
7. Observe the flask after 24 hours and replace the medium with fresh medium.
Note: It is recommended to analyze the cells for stem cell markers by immunostaining and / or flow cytometry after revival and assess the potency of the cells to differentiate into different lineages.

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 dilute the cryoprotectant slowly 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.
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
Yellow to brown coloured clear solution.

pH
7.00 -7.60

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 stem cells and assessing viability after thawing and comparing it with a control medium.

Storage and Shelf Life:

Cell Freezing Media should be stored at -20°C. For frequent use, cell freezing medium once thawed can be stored at 2-8°C up to 4 weeks. Avoid repeated freeze-thaw cycles.
Use before expiry date given on the product label.

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