

## Product Information

# Cell Freezing Media

Sterile-filtered, Suitable for cell culture

**C6039, C6164, C6295**

Storage Temperature -20 °C

## Product Description

The Cell Freezing Media are complete ready-to-use reagents which are designed to protect and preserve cells during frozen storage.

The Cell Freezing Medium-Glycerol 1× (C6039) and Cell Freezing Medium-DMSO 1× (C6164) contain Minimum Essential Medium (MEM) supplemented with a mixture of fetal bovine serum and calf serum and contain 10% glycerol or DMSO.

The Cell Freezing Medium-DMSO Serum free 1x (C6295) is a serum-free preparation prepared according to the original published formula of Charity Waymouth.<sup>1</sup> It contains 8.7% DMSO in MEM supplemented with methyl cellulose.

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

Cell Freezing Media should be stored at -20 °C. After thawing, these products may be stored at 2-8 °C for up to 5 days. It is not recommended to store reagents in a frost-free freezer as temperature cycling may cause deterioration of the products.

## Procedure

This product does contain phenol red. Due to shipment on dry ice, there could be significant carbon dioxide buildup in the package. This CO<sub>2</sub> may enter the solution and lower the pH slightly, giving an orange rather than red color. The color will typically return to its red color when thawed. Loosening the cap and briefly placing the bottle in a hood or incubator and allowing the solution to expel any excess CO<sub>2</sub> can also help the solution return to the normal red color.

## Cell Freezing Media

It may be used in standard freezing protocols. The following protocol may be used:

1. Thaw the Cell Freezing Medium and hold it on wet ice.
2. Remove adherent cells with trypsin or other appropriate means. For optimal results cells should be in the log phase of growth.
3. Gently pellet (10 minutes at 250 x g at 2-8 °C) the cells by centrifugation and remove as much of the growth medium as possible.
4. Suspend the cells in freezing medium at 10<sup>6</sup> to 10<sup>7</sup> cells/mL. Myelomas or hybridomas may require a higher cell density.
5. Aliquot cells into freezing vials, holding them on wet ice until freezing begins (within 5 minutes).
6. Freeze cells according to standard protocols. Store below –70 °C.

## Thawing of frozen cells

1. Remove cells from frozen storage and quickly thaw in a 37 °C water bath.
2. Dilute 1 mL of cell suspension with 10 mL of complete growth medium.
3. Gently mix and pellet cells by gentle centrifugation.
4. Suspend cells in complete growth medium and plate in appropriate vessels.

## References

1. Waymouth, C., and Varnum, D., (1976). Simple Freezing Procedure for Storage in Serum-Free Media of Cultured and Tumor Cells of Mouse. TCA Manual 2:1 pp. 311-313.
2. Freshney, R., (1986). Animal Cell Culture: A Practical Approach. IRL Press Ltd., Oxford. pp. 73-77.
3. Kruse, P. and Patterson, M. (1973). Tissue Culture Methods and Applications. Academic Press, NY. pp. 712-718.

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