Highly Efficient, Safe, and Easy-to-use Freezing Media Optimized for Cell Therapy

- FDA Drug Master File (DMF) Supported
- USP Grade Components
- Manufactured under cGMP Conditions
- Pre-Formulated with DSMO
- Serum-Free & Protein-Free
- Animal-Derived Component-Free
- Chemically Defined
- Aseptic Processing Technique
- Low Endotoxin
- Cell-Based Release Testing

Test methods and criteria are provided on all lot-specific Certificates of Analysis (COA) and release.

Cat. No.	Product	Size
AR0008-100	Cell Freezing Media - CGT	100mL/Bottle
AR0008-100B	Cell Freezing Media - CGT	100mL/Bag

Cryopreservation Protocol

1. Cell Processing: Isolate cells to be cryopreserved into suspension using mechanical or enzymatic dissociation. Rinse cells once with an appropriate buffer (e.g.,DPBS) to remove the residual enzyme or separation medium.
2. Cell Counting:. Count the total cell number prior to cryopreservation to calculate the required volume of Cell Freezing Media – CGT.
3. Centrifuge cells to obtain a compact cell pellet.
4. Remove Supernatant: Remove the supernatant (typically residual buffer) as much as possible to minimize the dilution of Cell Freezing Media-CGT.
5. Add cold (2~8°C) Cell Freezing Media-CGT to resuspend the cells.
   a) Recommended Cell Density: 5×10^5 cells/mL - 5×10^7 cells/mL (Higher cell density might be feasible, but subject to further validation).
   b) Kryogene® Cell Freezing Media-CGT is pre-formulated with DMSO, and no additional additives are required.
   - Note 1: Add Cell Freezing Media-CGT dropwise to minimize rapid change between intracellular and extracellular osmotic pressure, which may cause cell damage.
   - Note 2: Pre-cool Cell Freezing Media-CGT at 2~8°C prior to use.
6. Aliquot the cell suspension into cryotubes or cryobags.
7. Pre-freeze Incubation: Incubate the cell suspension at 2~8°C for approximately 10 minutes prior to freezing.
8. Controlled Freezing: Freeze samples at -80°C (±10°C).
   a) Use a controlled rate freezer (-1°C/min) or similar protocol for most mammalian cells.
   b) The freezing device or isopropanol container should be pre-cooled to 2~8°C prior to use.
   c) The recommended freezing duration at -80°C using isopropanol containers is approximately 4

hours, with a maximum limit of overnight.

d) The controlled-rate freezer should be validated to ensure optimal cryopreservation.

9. Cell Storage: Transfer cryovials or cryobags to a liquid nitrogen environment for storage.

a) Cells should be placed in liquid nitrogen temperature (-196℃) for long-term storage.

b) Storage at -80℃ is only recommended for short-term use (weeks to months).

10. Cell Thawing: Rapidly thaw the cells in a 37℃ water bath or an equivalent mechanical thawing device.

a) Immediately transfer samples to a 37℃ water bath or equivalent mechanical thawing device after removal from liquid nitrogen. Avoid prolonged exposure to room temperature.

b) Gently agitate the cryovial or cryobag during thawing. Remove the container from the warming device while a small amount of ice remains. **Do Not** over-thaw.

c) When the cryotube or cryobag is removed from the warming device, it should be cooler than body temperature.

11. Dilute the cells/Cell Freezing Media-CGT mixture with culture medium or equivalent isotonic solution immediately after thawing.

a) The temperature for the dilution medium should be between 20℃ and 37℃.

b) Add the dilution buffer to the mixture slowly to prevent cell swelling or lysis caused by a sudden drop in osmotic pressure

c) It is recommended that the dilution ratio is no less than 1: 10 (Sample: buffer).

12. Measure the post-thaw viability and number of cells and place cells into culture conditions or use them immediately.

Note:

1. The long-term storage condition for Kryogene® Cell Freezing Media-CGT is 2~8℃, protect from light.

2. For further technical support, please contact us via Info@milecell-bio.com.

One Innovation Drive Suite 420,
Worcester, MA 01605, U.S.

+1(351)-215-8296
Info@milecell-bio.com
www.milecell-bio.com