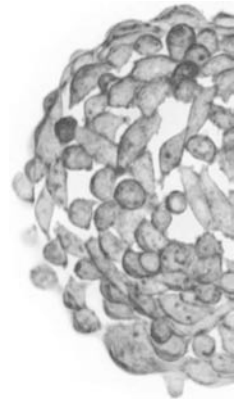


Global Cell Solutions Culture. Simplified.



Protocol: Freezing Cells on the GEM

Questions? Suggestions? We want to help! Please contact us at:
+1-434-975-4271, ext 103
support@globalcellsolutions.com

This is a general method for freezing cells cultured on the GEM. Adjust volumes suggested accordingly for the culture format and vessel you are using.

Materials:

- Cells on GEM
- DMSO Dimethyl Sulfoxide (10% v/v)¹
- Fetal Bovine Serum (FBS)
- Pre-labeled cryogenic vials (2mL vials)
- BioMagnet
- Ice: It's easier to follow this protocol if you have the ice sitting in a flat container or bucket for easy placement of plates or LeviTubes and vials. It is recommended that most steps of this protocol be executed over ice.²

1. Prepare 10mL of 'DMSO Freezing Media Wash' (5% DMSO, 20% FBS):
 - a. Combine 0.5mL DMSO, 2mL of FBS, and 7.5mL of culture media.
 - b. Place on ice.

¹ The DMSO we have been successful using was purchased from Sigma-Aldrich (Catalog No. D2650).

² DMSO will cause the cells to detach from the GEM™ substrate when it's at room temperature. Keep all cells on GEM, freezing media, and wash solutions ICE cold.

2. Prepare 5mL 'Freezing Media Wash' (20% FBS):
 - a. Combine 1mL of FBS and 3.5mL of culture media.
 - b. Place on ice.
3. Place the cells on GEM in the tissue culture hood. Distribute 2-4 million cells per 2mL cryovial.
4. Using the BioMagnet, pull the confluent GEM to the bottom of the cryovial. Aspirate all media, taking care not to disturb the GEM layer.
5. Gently add in 900µl of the 'DMSO Freezing Media Wash'. Mix gently.
6. Using the BioMagnet, pull the cells on GEM to the bottom of the cryovial. Aspirate the 'DMSO Freezing Media Wash', taking care not to disturb the GEM layer.
7. Repeat step 6.
8. Gently add 900µl of 'Freezing Media Wash'.
9. With a 200µl pipette, draw up 100µl DMSO. Add 1 drop of DMSO into the cryovial and swirl. Then add in 2 drops and swirl. Continue in this fashion (doubling the number of drops used) until all 100µl of DMSO are used. The final concentration of DMSO in the tube will be 10%, in 1mL total volume per 2mL cryovial.
10. Freeze according to ATCC Technical Bulletin No. 3, *Cryogenic Preservation of Animal Cells*³.

³ <http://www.atcc.org/common/technicalInfo/TechLit.cfm> (viewed on December 3, 2005)