



ESGRO COMPLETE™ SERUM-FREE CELL CULTURE FREEZING MEDIUM

CATALOG NUMBER: SF005

QUANTITY: 50 mL

LOT NUMBER:

DESCRIPTION: ESGRO Complete Serum-free Cell Culture Freezing Medium is qualified for use with mouse embryonic stem cells cultured in serum-free conditions with ESGRO Complete PLUS Clonal Grade Medium (Cat. Nos. SF001-100P or SF001-500P). The optimized formulation allows for consistent cryopreservation and high viability upon thawing.

APPLICATIONS: Cryopreservation of mouse ES cells cultured in serum-free conditions

PROTOCOL:

1. Thaw cell culture freezing medium completely and mix well by gently swirling bottle. Keep freezing medium on ice during use.
2. Cells to be frozen should be in late log phase growth.
3. Monolayers will need to be dissociated. After dissociation, cells are resuspended in serum-free Clonal Grade Medium and counted to determine viability and number.
4. Centrifuge cells at 1300 rpm for 3 min. Remove the medium above the pellet.
5. Resuspend the cells in cell culture freezing medium at a concentration of $\sim 4 \times 10^6$ cells/mL. Freeze 1 mL of cells/vial. After the cells have been resuspended and aliquoted into appropriate cryogenic storage vials, they can be placed on dry ice and your normal freeze down procedure should be started within five minutes.
6. Cells must be stored at or below -80°C . For long term storage the cells should be stored in ultra-low temperature freezers (-150°C), or in liquid nitrogen (-196°C).
7. Thawing of cryopreserved cells should be as follows:
 - a. Thaw cells quickly in a 37°C water bath.
 - b. Dilute one vial of cells into 10 mL of prewarmed Clonal Grade Medium.
 - c. Gently mix the cells in the growth media.
 - d. Gently pellet cells and remove the medium above the pellet.
 - e. Resuspend the cells in Clonal Grade Medium, dilute to the appropriate concentration, and plate into the appropriate vessel.

FORMAT: Serum-free formulation. Contains 10% DMSO and BSA.

STORAGE/HANDLING: Store at -20°C . Refer to lot expiration date on label.

REFERENCES: Ying, Q., Nichols, J., Chambers, I., Smith, A. (2003). BMP Induction of Id Proteins Suppresses Differentiation and Sustains Embryonic Stem Cell Self-Renewal in Collaboration with STAT3. *Cell* **115**:281-292.

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