

## ESGRO COMPLETE™ C57/BL6 MOUSE EMBRYONIC STEM CELL LINE

<b>CATALOG NUMBER:</b>	SF-CMTI-2	<b>QUANTITY:</b>	5 x 10 <sup>6</sup> cells, passage 12
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2.5 x 10 <sup>6</sup> cells / vial
<b>DESCRIPTION:</b>	ESGRO Complete C57/BL6 mouse ES cell line has been pre-adapted to serum-free and feeder-free cell culture conditions and is intended for use with the ESGRO Complete cell culture system. This mouse ES cell line has been confirmed germline competent.		
<b>STRAIN:</b>	C57/BL6		
<b>KARYOTYPE:</b>	90% normal male mouse karyotype		
<b>PATHOGEN TESTING:</b>	Cells tested negative for mycoplasma and other pathogens by infectious disease PCR.		
<b>PRESENTATION:</b>	Cells are supplied frozen in ESGRO Complete Freezing Medium		
<b>STORAGE/HANDLING:</b>	Place vials in the vapor phase of liquid nitrogen storage immediately upon receipt until it is convenient to proceed to subculture.		

<b>MATERIALS REQUIRED BUT NOT SUPPLIED:</b>	<b>Description</b>	<b>Volume</b>	<b>Catalog #</b>
	ESGRO Complete Clonal Grade Medium	500 mL	SF001-500
	ESGRO Complete Basal Medium	500 mL	SF002-500
	ESGRO Complete Freezing Medium	50 mL	SF005
	Accutase™ Cell Dissociation Solution	100 mL	SF006
	ESGRO Complete Gelatin	500 mL	SF008
	EmbryoMax® D-PBS, w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>	500 mL	BSS-1006-B

**CULTURE NOTES:** It is recommended that the serum-free adapted C57/BL6 murine ES cells are cultured in ESGRO Complete Clonal Grade Medium. Optimal results are achieved when the cells are fed daily and passaged 1:3 to 1:4 when reaching 60% confluence.

- Incubator settings: 7.5% CO<sub>2</sub> in humidified air, 37°C
- Replace the media daily (please refer to the recommended media below).
- Culture dishes need to be gelatin coated prior to use
- Use Accutase solution to passage the cells

**PREPARATION OF CULTURE DISH:**

Gelatin coating protocol – Serum Free Media

1. Warm ESGRO Complete 0.1% Gelatin Solution to room temperature before use.
2. Add enough Gelatin Solution to adequately cover plasticware surface.
3. Leave with lid on in laminar flow hood for 15 minutes.
4. Aspirate Gelatin Solution.
5. Add media and cell suspension to plasticware.

**THAWING CELLS:**

Thawing of 1 vial of C57/BL6 ES cells into a 25 cm<sup>2</sup> flask.

1. Prepare a 25 cm<sup>2</sup> flask with ESGRO Complete Gelatin Solution.
2. Equilibrate Clonal Grade Medium in a 37°C water bath 1 hour before thawing the ES cells
3. Thaw one vial of C57/BL6 ES cells by gently shaking the tube in a 37°C water bath. When the contents of the tube have thawed, spray the vial with ethanol, dry the outside of vial, and aseptically transfer the contents of the vial to a tube containing 5 mL of pre-warmed Clonal Grade Medium
4. Centrifuge for 3 min at 1300 rpm
5. Aspirate the medium and resuspend the cell pellet in 8 mL of Clonal Grade Medium
6. Transfer the cell suspension to the gelatin coated 25 cm<sup>2</sup> flask
7. Place the flask in a 37°C incubator.

**LOW DENSITY PLATING ASSAY:**

1. Plate ES cells onto gelatin coated 10 cm plastic tissue culture dish at 1000 cells/10 cm dish in 10-20 mL of Clonal Grade Medium.
2. Change medium every 2-4 days.
3. Monitor for clonal growth with alkaline phosphatase stain.
4. Colony formation should occur over 4-6 days. The majority of colonies should be alkaline phosphatase positive and show no signs of differentiation.

**PASSAGING CELLS:**

Passage of the C57/BL6 ES cells from a 25 cm<sup>2</sup> flask to a 75 cm<sup>2</sup> flask.

1. Prepare a 75 cm<sup>2</sup> flask with 0.1% Gelatin Solution.
2. Examine the ES cells under the microscope. The cells should be 60% confluent.
3. Thaw Accutase solution to room temperature.
4. Equilibrate Clonal Grade Medium in a 37°C water bath 1 hour before passaging the ES cells
5. Aspirate the medium from the 25 cm<sup>2</sup> flask containing the C57/BL6 ES cells and rinse with 5 mL of PBS.
6. Aspirate the PBS and add 1 mL of Accutase solution to the flask using aseptic procedures
7. Return culture to 37°C incubator and allow cells to detach (3-5 minutes).
8. Tap flask to remove all colonies. Add 5 mL of pre-warmed Basal Medium and gently make a cell suspension by pipetting up and down.
9. Count cells if necessary and centrifuge 3 min at 1300 rpm.
10. Remove supernatant and add again 5 mL of Basal Medium, mix and spin.
11. Resuspend in 15 mL of pre-warmed Clonal Grade Medium.
12. Transfer the cell suspension to the prepared 75 cm<sup>2</sup> flask.
13. Place the flask in the 37°C incubator.

**Important Application Notes:** When passing cells in Accutase, incubate no more than 5-10 minutes or the least amount of time and resuspend cells to a single cell suspension, as otherwise the cells tend to clump together to form floating colonies. Compared to regular trypsin, Accutase requires more rigorous resuspension.

It is important keep the flask sub-confluent (about 60%), as mES cells tend to differentiate in more confluent conditions.

When seeding mES cells, as a general rule stay within  $1-2 \times 10^6$  cells per T25 flask. It may be necessary to count cells during some passages.

#### 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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