

ESGRO COMPLETE™ C57/BL6 MOUSE EMBRYONIC STEM CELL LINE

CATALOG NUMBER:	SF-CMTI-2	QUANTITY:	5 x 10 ⁶ cells, passage 12
LOT NUMBER:		CONCENTRATION:	2.5 x 10 ⁶ cells / vial
DESCRIPTION:	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.		
STRAIN:	C57/BL6		
KARYOTYPE:	80% normal male mouse karyotype		
PATHOGEN TESTING:	Cells tested negative for mycoplasma and other pathogens by infectious disease PCR.		
PRESENTATION:	Cells are supplied frozen in ESGRO Complete Freezing Medium		
STORAGE/HANDLING:	Place vials in the vapor phase of liquid nitrogen storage immediately upon receipt until it is convenient to proceed to subculture.		

MATERIALS REQUIRED BUT NOT SUPPLIED:	Description	Volume	Catalog #
	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 ²⁺ & Mg ²⁺	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% CO₂ 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 cm² flask.

1. Prepare a 25 cm² 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 cm² 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 cm² flask to a 75 cm² flask.

1. Prepare a 75 cm² 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 cm² 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 cm² 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 x10⁶ 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.

PRODUCT LICENSE

The purchase of this product conveys to the purchaser the non-transferable right to use the cells for research use only conducted by the purchaser. For commercial purchasers using this product for research, the purchase of this product conveys to the purchaser the non-transferable right to use the purchased cells for a six (6) month evaluation period after the date of purchase for research use only. After the six (6) month evaluation period, the purchaser must contact Millipore Corporation or its designee to obtain a commercial license.

The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for any purpose. The purchaser cannot use this product or its components in whole plants, plant cells or humans.

Commercial purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is not willing to accept the limitations of the limited use license, Millipore is willing to accept return of the products with a full refund. For information on commercial use of this product or obtaining a license to this product for purposes other than research, contact the Millipore Corporation Intellectual Property Department.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

©2008: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.