



OVERVIEW

Mouse Embryonic Stem Cell Culture

Cat. Numbers:

ASE-9005	ASM-5001	ASM-5007
ASE-9006	ASM-5003	ASM-5009
ASE-9007	ASM-5006	ASM-5011

Culture Procedure

1. Preparation of Mouse Embryonic Stem Cell (mESC) culture medium

- Prepare mESC medium either by (A) mixing ESC-Sure™ mESC Mate (#ASM-5009) with ESC-Sure™ DMEM (#ASM-5001) or (B) preparing it from stock solutions according to tables.
- Keep sterile, store at 4°C in the dark and use within 14 days

(A) ESC-Sure™ mESC Mate-based mESC medium

Component	Cat #	Final conc.	100 ml	500 ml
ESC-Sure™ DMEM	ASM-5001		80 ml	400 ml
ESC-Sure™ Mate	ASM-5009 ASM-5011	20 %	20 ml	100 ml

(B) Alternative mESC medium

Component	Cat #	Stock conc.	Final conc.	100 ml	500 ml
ESC-Sure™ DMEM	ASM-5001			77 ml	385 ml
ESC-Sure™ FBS	ASM-5006 ASM-5007		20 %	20 ml	100 ml
Sodium Pyruvate		100 mM	1 mM	1 ml	5 ml
L-Glutamine		200 mM	2 mM	1 ml	5 ml
Non-essential amino acids		10 mM	100 µM	1 ml	5 ml
BME		1.43 M	100 µM	0.7 µl*	3.5 µl
LIF			0.2 %	200 µl	1 ml

* For preparation of a small amount of medium, BME can be diluted 1:10 in PBS. This dilution however is not stable and should be prepared fresh.

2. Preparation of MEF (mouse embryonic fibroblast) feeder cells

- Plate mitotically inactivated MEF cells in MEF culture medium on a culture dish at the density of 30,000 - 50,000 cells/cm² (please refer to our MEF protocol and datasheets)

3. Thawing mouse ES cells

- Take a vial from the liquid nitrogen tank and immediately place it into 37°C water bath.
- As soon as most of the medium is melted, transfer cells into a 15 ml tube with warm culture medium and centrifuge at 1000 rpm for 5 min.
- Resuspend pellet in mESC medium and plate onto MEF feeder cells

4. Routine Culture

- Check the mESC culture under the microscope and change the medium daily

5. Passaging mouse ES cells (every 2- 3 days)

- Aspirate the medium and wash cells with PBS
- Add prewarmed trypsin and incubate at 37°C for 5 minutes
- Use the same volume of mESC medium to neutralize the trypsin
- Transfer into 15 ml centrifuge tube and spin at 1000 rpm for 5 minutes
- Aspirate the supernatant, resuspend cells in fresh medium and plate onto fresh MEF cells

6. Freezing mouse ES cells

- Change medium 2-3 hours before freezing ES cells
- Remove culture medium and wash mES cells with PBS
- Add prewarmed trypsin and incubate at 37°C for 5 minutes
- Use the same volume of mESC medium to neutralize the trypsin
- Transfer into 15 ml centrifuge tube and spin at 1000 rpm for 5 minutes
- Remove the supernatant and resuspend the pellet in the appropriate volume of mESC culture medium (0.5 ml per vial)
- While gently tapping the tube, very slowly and drop-wise add an equal volume of cold 2x Mouse ES cell Freezing Medium (#ASM-5003)
- Transfer cell suspension into labeled cryovials that are pre-cooled (1 ml per vial)
- Place at -80°C overnight

Applied StemCell, Inc.,
1165 O'Brien Dr, Suite A
Menlo Park, CA 94025

Phone: (866) 497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com

- After 24 hours transfer cryovials to liquid nitrogen for long-term storage

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