invitrogen™

GIBCO® Mouse Embryonic Fibroblasts (Irradiated)

Catalog nos. S1520-100, S1520-250

Revision date: 9 February 2010
Manual part no. A11560

MAN0001756

User Manual

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Contents and Storage

Kit Configurations

Catalog no. S1520-100 includes cells only.

Catalog no. S1520-250 includes cells plus media.

Shipping

GIBCO® Mouse Embryonic Fibroblasts (Irradiated) and KnockOut™ Serum Replacement are shipped on dry ice.

Kit Contents and Storage

Kit components and storage conditions for S1520-100 and S1520-250 are listed in the table below.

S1520-100	Amount	Storage
GIBCO® Mouse Embryonic Fibroblasts (Irradiated) (1×10^6 cells/mL in freezing medium*)	1 mL	Liquid nitrogen
S1520-250	**Amount**	**Storage**
GIBCO® Mouse Embryonic Fibroblasts (Irradiated) (1×10^6 cells/mL in freezing medium)	1 mL	Liquid nitrogen
KnockOut™ Serum Replacement	100 mL	–5 to –20°C

***Freezing medium:** 60% Dulbecco's Modified Eagle medium containing 4.5 g/L glucose, 30% Fetal Bovine Serum, and 10% DMSO.

Handle cells as potentially biohazardous material under at least Biosafety Level 1 (BL-1) containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Safety Data Sheet (SDS) before handling. Safety Data Sheets (SDSs) are available on our website at www.invitrogen.com/sds.

Intended Use

GIBCO® Mouse Embryonic Fibroblasts (Irradiated) are for research use only. They are not intended for any animal or human therapeutic or diagnostic use.

GIBCO® Mouse Embryonic Fibroblasts (Irradiated)

Uses of GIBCO® Mouse Embryonic Fibroblasts (Irradiated)

GIBCO® Mouse Embryonic Fibroblasts (Irradiated) are used as feeder layers for culturing embryonic stem cells (ESCs), including mouse and human ESCs, in their undifferentiated state. The growth-arrested feeder layer supports the ESC culture by providing nutrients, growth factors, and matrix components, and it enables ESCs to survive and proliferate more readily in culture.

Source of GIBCO® Mouse Embryonic Fibroblasts (Irradiated)

GIBCO® Mouse Embryonic Fibroblasts (Irradiated) were isolated from ICR mouse embryos at day 13.5 of gestation under sterile conditions, expanded for up to two passages in D-MEM medium containing 4.5 g/L glucose supplemented with 10% FBS, and mitotically inactivated by γ-irradiation. After γ-irradiation, cells were cryopreserved in a cryopreservation medium composed of 60% D-MEM (high glucose), 30% FBS, and 10% DMSO. Each vial of GIBCO® Mouse Embryonic Fibroblasts (Irradiated) contains approximately 1×10^6–1.5×10^6 live cells.

Characteristics of GIBCO® Mouse Embryonic Fibroblasts (Irradiated)

- Isolated from mouse primary cell cultures
- Mitotically inactivated by γ-irradiation
- Frozen at passage number up to 2 (≤ P2)
- Support the growth of embryonic stem cells (ESCs), including mouse and human ESCs in their undifferentiated state

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Guidelines for Using MEFs

Follow the guidelines below to use inactivated mouse embryonic fibroblasts (MEFs) as feeder layers to culture mouse and human embryonic stem cells (ESCs).

- **All solutions and equipment that come in contact with the cells must be sterile.** Always use proper aseptic technique and work in a laminar flow hood.
- Make sure to start preparing MEF feeder layers two days before culturing ESCs.
- After thawing, transfer MEFs into pre-warmed medium.
- Plate MEFs on culture vessels coated with 0.1% gelatin solution (see page 3).
- Use MEF dishes or plates within one week after preparation.
- Before starting experiments, ensure that ESCs have been established (at least 1 passage), and also have some frozen ESC stocks on hand.

Media Requirements

For best results, we recommend using Dulbecco's Modified Eagle medium (D-MEM) containing 4.5 g/L glucose, and supplemented with 10% FBS for establishing GIBCO® Mouse Embryonic Fibroblasts (Irradiated) (see page 22 for ordering information). Prepare the medium prior to use.

As with other mammalian cell lines, when working with GIBCO® Mouse Embryonic Fibroblasts (Irradiated), handle as potentially biohazardous material under at least Biosafety Level 1 (BL-1) containment. For more information on BL-1 guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., published by the Centers for Disease Control, which is available for downloading at: www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

Culturing MEF Feeder Cells

Preparing Medium and Culture Vessels

Materials Needed

For gelatin coating culture vessels:

- AF solution (Attachment Factor)

  Note: AF is a sterile 1X solution containing 0.1 % gelatin available from Invitrogen (see page 22 for ordering information).

- Sterile culture vessels

For preparing MEF medium:

- Dulbecco's Modified Eagle medium (D-MEM) containing 4.5 g/L glucose
- Fetal bovine serum (FBS), ES-Cell Qualified

Gelatin Coating Culture Vessels

1. Cover the whole surface of each culture vessel with AF solution and incubate the vessels for 30 minutes at 37°C or for 2 hours at room temperature.
2. Using sterile technique in a laminar flow culture hood, completely remove the AF solution from the culture vessel by aspiration.

   Note: It is not necessary to wash the culture surface before adding cells or medium.

3. Coated vessels may be used immediately or stored at room temperature for up to 24 hours.

Preparing MEF Medium

1. Prepare 500 mL of MEF medium by mixing the following components (pre-warmed to 37°C):

D-MEM	450 mL
FBS	50 mL

Thawing and Establishing MEFs

Materials Needed

- GIBCO® Mouse Embryonic Fibroblasts (Irradiated), frozen
- MEF medium (see previous page)
- Gelatin coated culture vessels (see previous page)
- Phosphate buffered saline (PBS) without Ca^{2+} or Mg^{2+}
- 37°C water bath
- 70% ethanol
- Disposable, sterile 0.5 mL and 15-mL tubes
- Microcentrifuge
- Hemacytometer, cell counter and Trypan Blue, or the Countess™ Automated Cell Counter

Thawing MEFs

1. Remove the cryovial containing inactivated MEFs from the liquid nitrogen storage tank.
2. Briefly roll the vial between hands to remove frost, and swirl it gently in a 37°C water bath.
3. When only a small ice crystal remains in the vial, remove it from water bath. Spray the outside of the vial with 70% ethanol before placing it in the cell culture hood.
4. Pipet the thawed cells gently into a 15-mL conical tube.
5. Rinse the cryovial with 1 mL of pre-warmed MEF medium. Transfer the medium to the same 15-mL tube containing the cells.
6. Add 4 mL of pre-warmed MEF medium **dropwise** to the cells. Gently mix by pipetting up and down.
   Note: Adding the medium slowly helps the cells to avoid osmotic shock.
7. Centrifuge the cells at 200 × g for 5 minutes.
8. Aspirate the supernatant and resuspend the cell pellet in 5 mL of pre-warmed MEF medium.
9. Remove 20 µL of the cell suspension and determine the viable cell count using your method of choice.

Note

Invitrogen's Countess™ Automated Cell Counter is a benchtop counter designed to accurately measure cell count and viability in less than a minute per sample, using the standard Trypan Blue technique (see page 23 for ordering information).

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Plating MEFs

1. Centrifuge the remaining cell suspension (step 9, previous page) at 200 × g for 5 minutes at room temperature.
2. Aspirate the supernatant. Resuspend the cell pellet in MEF medium to a density of 2.5×10^6 cells/mL.
3. Aspirate the gelatin solution from the gelatin coated culture vessel (step 4, page 3), and wash the vessels once with PBS.
4. Add the appropriate amount of MEF medium into each culture vessel (refer to the table below).
5. Into each of these culture vessels, add the appropriate amount of MEF suspension (refer to the table below).

   Note: The recommended plating density for GIBCO® Mouse Embryonic Fibroblasts (Irradiated) is **2.5×10^4 cells/cm^2**.
6. Move the culture vessels in several quick back-and-forth and side-to-side motions to disperse the cells across the surface of the vessels.
7. Incubate the cells in a 37°C incubator with a humidified atmosphere of 5% CO_2.
8. Use the MEF culture vessels within 3–4 days after plating.

Vessel Size	Growth Area	Volume of Media	Number of MEFs	Volume of MEF Suspension
96-well plate	0.32 cm^2/well	0.1 mL	1.0×10^4/well	4 µL
24-well plate	2 cm^2/well	0.5 mL	5.0×10^4/well	20 µL
12-well plate	3.8 cm^2/well	1 mL	1.0×10^5/well	40 µL
6-well plate	9.6 cm^2/well	2 mL	2.5×10^5/well	0.1 mL
60-mm dish	19.5 cm^2	5 mL	5.0×10^5	0.2 mL
100-mm dish	58.95 cm^2	10 mL	1.5×10^6	0.6 mL
25-cm^2 flask	25 cm^2	5 mL	6.3×10^5	0.25 mL
75-cm^2 flask	75 cm^2	15 mL	1.9×10^6	0.75 mL

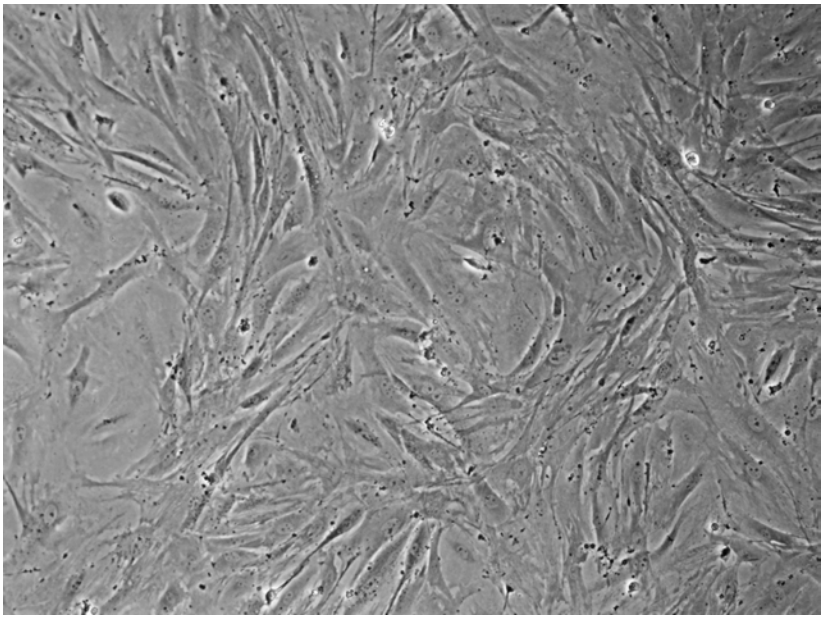
Important

The number of cells and the volume of cell suspension given in the table above are optimized **for MEFs only**. For plating other cell types (e.g., mESCs), calculate the number of cells to be plated using the growth area and the recommended plating density for the specific cell type you are using.

Expected Results

Images of MEF Culture

The bright field image below shows GIBCO® Mouse Embryonic Fibroblasts (Irradiated) plated at the recommended density on culture dishes coated with 0.1% gelatin. The image was taken with a 10X objective.



Culturing Human ESCs

Preparing Media and Solutions

Materials Needed

- Basic fibroblast growth factor (bFGF), 10 µg/mL
- Phosphate buffered saline (PBS) without Ca^{2+} or Mg^{2+}
- 10% bovine serum albumin (BSA)
- D-MEM/F-12 with GlutaMAX™-I
- Knockout™ Serum Replacement (KSR)
- MEM Non-essential amino acids solution (NEAA)
- 2-Mercaptoethanol
- Dispase

Preparing Basic FGF Solution

1. To prepare 1 mL of 10 µg/mL basic FGF solution, mix the following components:

Basic FGF	10 µg
PBS	980 µL
10% BSA	10 µL

2. Aliquot and the solution store at –20°C for up to 6 months.

Preparing Dispase Solution

1. To prepare 50 mL of 2 mg/mL Dispase solution, mix the following components:

Dispase	100 mg
D-MEM/F-12	50 mL

2. Aliquot and the solution store at 4°C for up to 2 weeks or at –20°C for up to 6 months.

Preparing hESC medium

1. To prepare 100 mL of hESC medium, mix the components listed below. You can store the medium at 4°C for up to 7 days.

D-MEM/F-12	79 mL
KSR	20 mL
NEAA	1 mL

2. Before using the medium, add the components below and mix.

2-Mercaptoethanol	182 µL
Basic FGF	40 µL

Thawing and Establishing Human ESCs on MEF Feeder Cells

Materials Needed

- Human embryonic stem cells (hESCs)
- MEF culture vessels (step 7, page 5)
- hESC medium (see previous page)
- 70% ethanol
- Disposable, sterile 15-mL tubes
- 37°C water bath
- 37°C incubator with a humidified atmosphere of 5% CO_2
- Microcentrifuge
- Hemacytometer, cell counter and Trypan Blue, or the Countess™ Automated Cell Counter

Thawing hESCs

1. 3–4 hours before plating hESCs, aspirate the MEF medium from the 60-mm culture dish containing the MEFs and add 4 mL of hESC culture medium.

   Note: You will plate the hESC into this dish (see next page).
2. Remove a cryovial containing hESCs from the liquid nitrogen storage tank.
3. Roll the vial between your gloved hands briefly to remove frost, and swirl it gently in a 37°C water bath.
4. When only a small ice crystal remains in the vial, remove it from water bath. Spray the outside of the vial with 70% ethanol before placing it in the cell culture hood.
5. Pipet the thawed cells gently into a 15-mL conical tube.
6. Rinse the cryovial with 1 mL of pre-warmed hESC medium. Transfer the medium to the same 15-mL tube containing the cells (from step 5).
7. Add 4 mL of pre-warmed hESC medium **dropwise** to the cells. While adding the medium, gently move the tube back and forth to mix the hESCs.

   Note: Adding the medium slowly helps the cells to avoid osmotic shock.
8. Centrifuge the cells at 200 × g for 5 minutes at room temperature.
9. Aspirate the supernatant and resuspend the cell pellet in 5 mL of pre-warmed hESC medium.

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Thawing and Establishing hESCs on MEF Feeder Cells, continued

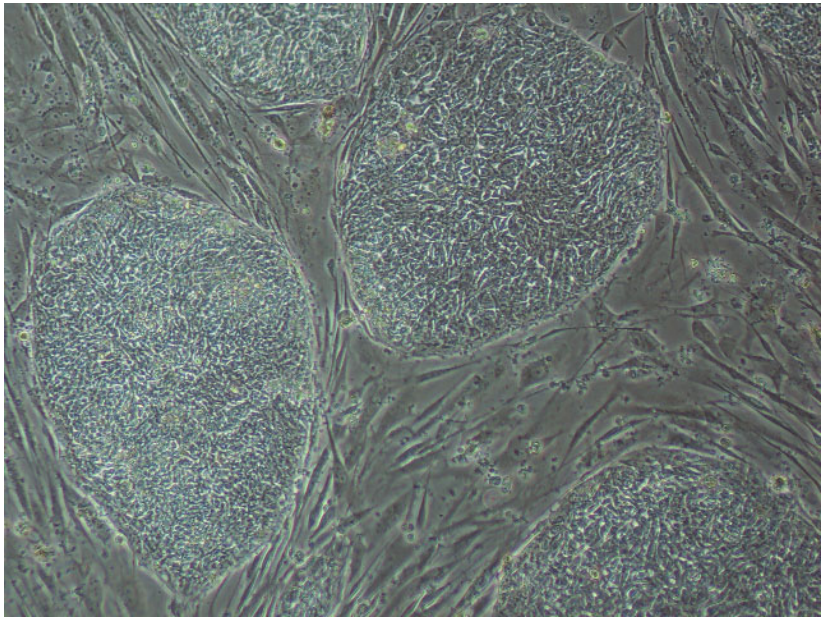
Plating hESCs

1. Aspirate the hESC medium from the 60-mm culture dish containing the MEFs (step 1, page 8).
2. Slowly add the hESC suspension into the MEF culture dish.
3. Move the vessel in several quick back-and-forth and side-to-side motions to disperse the hESCs across the surface of the dish.
4. Place the culture dish gently in a 37°C incubator with a humidified atmosphere of 5% CO_2.
5. Replace the spent medium and examine the cells under a microscope daily. It may take up to a week for colonies to become visible.
6. Observe the hESCs every day and passage them whenever the colonies are too big or crowded. The split ratio depends on the total number of hESCs in the culture dish (approximately 1:1 to 1:3 at the first passage after recovery).

Expected Results

Images of hESCs on MEF Feeder Cells

The image below shows H9 human embryonic stem cells (hESCs) cultured on a layer MEF feeder cells. The bright field image of the hESCs at passage 1 was obtained with a 10X.



Passaging Human ESCs on MEF Feeder Cells

Guidelines for Passaging hESCs

- Passage your hESCs when the first of the following occurs:
  - MEF feeder layer is two weeks old.
  - hESC colonies are becoming too dense or too large.
  - Increased differentiation occurs.
- The split ratio is varies, but it is generally between 1:4 and 1:6.
- Occasionally hESCs grow at a different rate, requiring the split ratio to be adjusted. A general rule is to observe the last split ratio and adjust the ratio according to the appearance of the hESC colonies.
- If the cells look healthy and colonies have enough space, split them using the same ratio as the previous passage; if they are overly dense and crowded, increase the ratio, and if the cells are sparse, decrease the ratio.
- Generally, hESCs need to be split every 5–7 days based upon their appearance.

Materials Needed

- Confluent hESCs growing on MEF feeder culture
- Fresh MEF culture vessels (see pages 3–5)
- hESC medium (see page 7)
- Dispase solution (see page 7)
- Disposable, sterile 15-mL tubes
- Cell scraper
- Dissecting microscope
- 37°C incubator with humidified atmosphere of 5% CO_2
- *Optional*: StemPro® EZPassage™ Disposable Stem Cell Passaging Tool (see **Note** on page 13)

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Passaging hESCs

1. Two days prior to passaging your hESC culture, prepare fresh MEF culture vessels following the instructions for MEF feeder cultures on pages 3–5.
2. 3–4 hours before plating the hESCs, aspirate the MEF medium from each MEF culture vessel, and add an appropriate amount of hESC medium (page 7) to each vessel according to the table on page 5.
3. Label the new MEF culture vessels with the cell line name, the new passage number, the date, the split ratio, and your initials. Return vessels into the incubator.
4. Remove the confluent hESC-MEF culture vessels from the incubator. Cut off the differentiated colonies under a dissecting microscope.
5. Aspirate the spent medium from the hESC-MEF culture vessels and add an appropriate amount of pre-warmed dispase solution to each hESC-MEF culture vessel (e.g., 2 mL to each 60-mm dish or 4 mL to each 100-mm dish).
6. Incubate the hESC-MEF culture vessels for 5–6 minutes at 37°C.
7. To confirm colony separation from the culture vessel, view the surface of the culture under a microscope. Look for the perimeter of the colonies to appear highlighted or folded back. The colonies will not be detaching from the surface completely.
8. Aspirate the dispase solution from the hESC-MEF culture vessels and add an appropriate amount of hESC medium to each vessel (e.g., 5 mL to each 100-mm dish).
9. Use a cell scraper to gently detach the hESCs off the surface of the vessels.
10. After the hESCs are detached from the surface of the culture vessel, pool the hESCs into a 15-mL centrifuge tube.
11. Rinse each hESC-MEF culture vessel with an appropriate amount of hESC medium. Transfer the medium to the same 15-mL tube containing the hESCs (from step 10).
12. Gently pipet the cells up and down a few times in the tube to further break-up the cell colonies.

Procedure continued on next page

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Passaging hESCs, continued

Procedure continued from previous page

13. Centrifuge the cells at 200 × g for 5 minutes at room temperature.
14. Aspirate the supernatant from the hESC pellet, and resuspend the pellet in an appropriate amount of hESC medium (e.g., 1–2 mL of medium for all the cells from one 60-mm dish).
15. Mix the cell suspension well using a pipette, being careful not to break up the colonies too much..
16. Add an appropriate amount of hESC suspension into each vessel containing MEFs according to the split ratio.
17. Move each culture vessel in several quick back-and-forth and side-to-side motions to disperse the cells across its surface. Return the culture vessels to the incubator after plating the hESCs.

    Note: While cells are attaching, open and close the incubator doors carefully. This will prevent disturbing the even distribution of cells on the surface of the vessels.
18. Incubate the cells overnight to allow the colonies to attach. Replace spent medium daily.
19. Observe hESCs every day and passage them whenever the colonies are too big or crowded (approximately every 5–7 days).

Note

hESCs cultured on MEF feeder layers can also be passaged using the StemPro® EZPassage™ Disposable Stem Cell Passaging Tool without the need for dispase treatment. For more information, refer to the product manual available at www.invitrogen.com.

Culturing Mouse ESCs

Thawing and Establishing Mouse ESCs on MEF Feeder Cells

Materials Needed

- Mouse embryonic stem cells (mESCs)
- MEF culture vessels (see page 5)
- MEF medium (see page 4)
- Knockout™ D-MEM
- Knockout™ Serum Replacement (KSR)
- MEM Non-essential amino acids solution (NEAA)
- 2-Mercaptoethanol
- Recombinant human LIF (leukemia inhibitory factor)
- L-Glutamine
- 70% ethanol
- Disposable, sterile 15-mL tubes
- 37°C water bath
- 37°C incubator with a humidified atmosphere of 5% CO_2
- Microcentrifuge
- Hemacytometer, cell counter and Trypan Blue, or the Countess™ Automated Cell Counter

Preparing mESC medium

1. To prepare 50 mL of mESC culture medium, mix the components listed below. You can store the medium at 4°C for up to 1 week.

Knockout™ D-MEM	41.5 mL
KSR	7.5 mL
NEAA	0.5 mL
L-Glutamine	0.5 mL

2. Before using the medium, add the components below and mix.

LIF (10 µg/mL)	50 µL
2-Mercaptoethanol	91 µL

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Thawing and Establishing mESCs on MEF Feeder Cells, continued

Thawing mESCs

1. Pre-warm the mESC medium (see previous page) to 37°C, and add 9 mL of mESC medium to a 15-mL conical tube
2. Remove a cryovial containing mESCs from liquid nitrogen storage and quickly thaw the vial in a 37°C water bath. Be careful not to submerge the entire vial.

   Note: Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. Thawing the cells for longer than 3 minutes results in decreased viability.
3. When the last ice crystal disappears, remove the vial from the water bath. Spray the outside of the vial with 70% ethanol before placing it in the cell culture hood.
4. Pipet the thawed cells gently into the 15-mL conical tube containing the pre-warmed mESC medium. Be careful not to introduce any bubbles.
5. Rinse the cryovial with 1 mL of pre-warmed mESC medium. Transfer the medium to the same 15-mL tube containing the cells (from step 4).
6. Centrifuge the cells at 250 × g for 5 minutes at room temperature.
7. Aspirate the supernatant and resuspend the cell pellet in an appropriate amount of pre-warmed mESC medium.
8. Remove 20 µL of the cell suspension and determine the viable cell count using your method of choice.

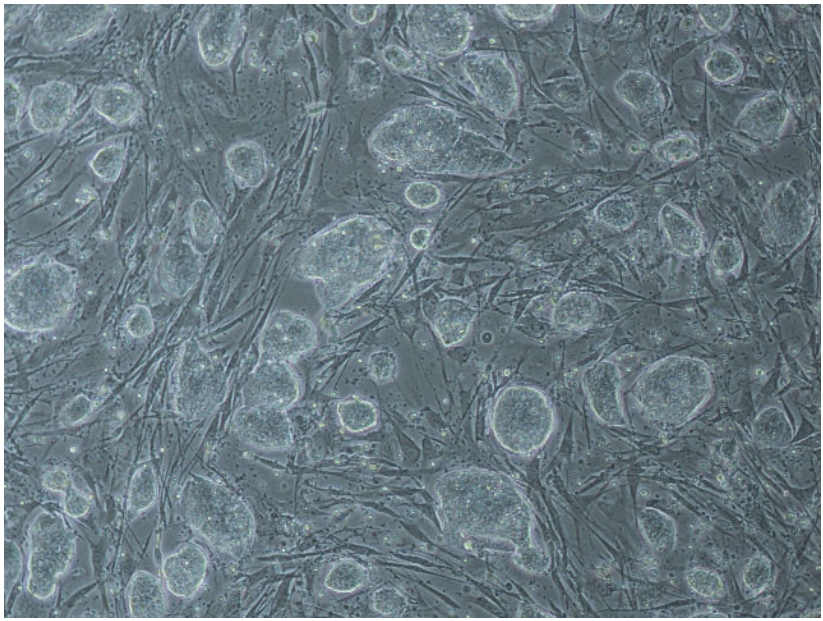
Plating mESCs

1. Aspirate the MEF medium from each culture vessel containing MEFs, and add an appropriate amount of mESC medium according to the table on page 5.
2. Plate the mESC suspension into MEF culture vessels at about **4×10^4 cells/cm^2**.
3. Add sufficient amount of mESC medium (e.g., a total of 2.5 mL of medium for each well of a 6-well plate). Gently rock the culture vessels to evenly distribute the cells.
4. Incubate the cells in a 37°C incubator with a humidified atmosphere of 5% CO_2.
5. Replace the spent medium with fresh pre-warmed mESC medium every day until mouse ESC colonies become confluent to be split.

Expected Results

Images of mESCs on MEF Feeder Cells

The images below show C57 mouse embryonic stem cells (mESCs) cultured on a layer MEF feeder cells. The bright field images of the mESCs were taken 2 days after plating with a 10X objective.



Passaging Mouse ESCs on MEF Feeder Cells

Guidelines for Passaging mESCs

- Plate mESCs at a density that provides an even distribution of colonies over the surface of culture vessel, but does not result in contact between the colonies. If colonies are plated too densely or too sparsely, they may differentiate.
- Do not over-passage mESCs from the C57BL/6 strain. Minimize the number of passages and the length of time the cells are kept in culture. This will ensure optimal and reproducible experimental results.
- Passage mESCs before the colonies become too large and dense. When plated at the optimal density, mESC should be passaged every 48 hours.
- Split ratios for mESCs can vary from 1:5 to 1:15.

Materials Needed

- Confluent mESCs growing on MEF feeder culture
- Fresh MEF culture vessels (see pages 3–5)
- mESC medium (see page 14)
- Phosphate buffered saline (PBS) without Ca^{2+} or Mg^{2+}
- StemPro® Accutase® Cell Dissociation Reagent
- Disposable, sterile 15-mL tubes
- 37°C incubator with humidified atmosphere of 5% CO_2
- *Optional*: StemPro® EZPassage™ Disposable Stem Cell Passaging Tool

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Passaging mESCs on MEF Feeder Cells,
continued

Passaging mESCs

1. Pre-warm the mESC medium, PBS, and Accutase® solution to 37°C.
2. Aspirate the spent MEF medium from each MEF culture vessel, and rinse the MEFs with PBS (3 mL for one well of a 6-well plate).
3. Aspirate the PBS from the MEF culture vessels and add an appropriate amount of pre-warmed mESC medium into the vessels (e.g., 2.5 mL for each well of a 6-well plate).
4. Return the MEFs to the 5% CO_2 humidified incubator.

   Note: Be careful not to disturb the monolayer of MEFs during steps 2–4.
5. Carefully aspirate the spent medium from culture vessels containing mouse ESCs, and rinse the cells with PBS (e.g., 3 mL for one well of a six-well plate).
6. Aspirate the PBS from the culture vessels.
7. Add an appropriate amount of Accutase® solution to cover the surface of culture vessels and incubate the vessels for 1–2 minutes until mESCs are dissociated. Gently tap the side of the culture vessels to detach the majority of cells from the surface of culture vessels.
8. Add mouse ESC medium (e.g., 3 mL for each well of a 6-well plate) to stop the dissociation reaction and gently pipet the cells up and down sufficiently to disperse the colonies into a single-cell suspension.

   Note: Be careful not to introduce any bubbles.
9. Transfer the mESC suspension into a 15-mL conical tube and centrifuge the tube at 250 × g for 5 minutes to pellet the cells.
10. Carefully aspirate as much of supernatant as possible and add an appropriate amount of mESC medium to the tube. Gently resuspend the mESCs.
11. Plate the mESCs into the culture vessels containing MEFs (step 4). Split ratios for mESCs can vary from 1:5 to 1:15.
12. Incubate mESCs at 37°C in a 5% CO_2 humidified incubator and change the medium every day. Mouse ESC can be split every other day.

Passaging Mouse ESCs using Mouse ESC Medium with FBS

Materials Needed

- Confluent mESCs growing on MEF feeder culture
- Fresh MEF culture vessels (see pages 3–5)
- Dulbecco's Modified Eagle Medium (D-MEM), low glucose
- Fetal Bovine Serum (FBS), ES-Cell Qualified
- MEM Non-essential amino acids solution (NEAA)
- 2-Mercaptoethanol
- Recombinant mouse LIF (leukemia inhibitory factor)
- Disposable, sterile 15-mL tubes
- Phosphate buffered saline (PBS) without Ca^{2+} or Mg^{2+}
- StemPro® Accutase® Cell Dissociation Reagent, Trypsin/EDTA Solution or TrypLE™ Express Dissociation Reagent
- Disposable, sterile 15-mL tubes
- 37°C incubator with humidified atmosphere of 5% CO_2
- *Optional*: StemPro® EZPassage™ Disposable Stem Cell Passaging Tool37°C water bath

Preparing mESC medium with FBS

1. To prepare 50 mL of mESC culture medium with FBS, mix the components listed below. You can store the medium at 4°C for up to 1 week.

D-MEM	44.4 mL
FBS	5 mL
NEAA	0.5 mL

2. Before using the medium, add the components below and mix.

Mouse LIF (10 µg/mL)	10 µL
2-Mercaptoethanol	91 µL

Passaging mESCs using mESC Medium with FBS

To passage mESC using mESC medium with FBS, follow the protocol on page 18, but replace mESC medium with mESC medium with FBS (see above). To detach the cells (Step 7 on page 18), you may use StemPro® Accutase® Cell Dissociation Reagent or Trypsin/EDTA solution (0.125% final concentration).

Troubleshooting

Culturing MEFs

The table below lists some potential problems and solutions that help you troubleshoot your MEF feeder cultures.

Problem	Cause	Solution
Cells have low viability after thawing	Stock not stored correctly	Order new stock and store in liquid nitrogen. Keep in liquid nitrogen until thawing.
	Thawing medium not correct	Use **pre-warmed** MEF medium, prepared as described on page 3.
	Cells too diluted	The recommended plating density for GIBCO® Mouse Embryonic Fibroblasts (Irradiated) is 2.5×10^4 cells/cm^2.
	Cell not handled gently.	GIBCO® Mouse Embryonic Fibroblasts (Irradiated) are fragile; treat your cells gently, do not vortex, bang the flasks to dislodge the cells, or centrifuge the cells at high speeds.
Cells not adherent after initial thaw	Did not use high-quality FBS.	Be sure to prepare your culture medium using ESC-qualified FBS (see page 22 for ordering information).

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ESC Culture on MEFs

The table below lists some potential problems and solutions that help you troubleshoot your embryonic stem cell (ESC) culture on MEF feeder layers.

Problem	Cause	Solution
No viable cells after thawing ESCs	Stock not stored correctly	Order new stock and store in liquid nitrogen. Keep in liquid nitrogen until thawing.
	Thawing medium not correct	Use **pre-warmed** ESC culture medium, prepared as described.
	Cell not handled gently	ESCs are fragile; treat your cells gently, do not vortex, bang the flasks to dislodge the cells, or centrifuge the cells at high speeds.
	Poor quality ESCs	ESCs were too old or overgrown when frozen. Obtain new stock.
Cells grow slowly	Growth medium not correct	Use **pre-warmed** ESC culture medium, prepared as described on page 7.
	Cells too old	Use healthy ESCs at low passage number; do not overgrow.
	Cells too diluted	Spin down cells for 4 minutes 200 × g at room temperature; aspirate media and plate cells at higher density.
	Basic FGF (bFGF) inactive	bFGF is not stable when frequently warmed and cooled. Add bFGF to medium just before use, or store medium with bFGF in aliquots at –20°C.
	Clump size is too small	Be gentle at time of passage so the clumps of cells don't get too small.
	Mycoplasma contamination	Discard cells, media and reagents, and use early stock of cells with fresh media and reagents.
Cells differentiated	Culture conditions not correct	Thaw and culture fresh vial of new ESCs. Follow thawing instructions and subculture procedures exactly.
	Cells too old	ESCs may become differentiated as their passage number increases.
	MEF feeder layer suboptimal	Prepare fresh MEF culture plates; follow the instructions on pages 3–5 exactly.
	Cells passaged too early	Passaging cells too early causes poor plating and differentiation. Grow cells to near-confluence.

Appendix

Accessory Products

Media, Sera, and Reagents

For more information about the following products, refer to www.invitrogen.com or contact Technical Support (see page 24).

Item	Quantity	Cat. no.
Dulbecco's Modified Eagle Medium (D-MEM), high glucose	500 mL	10569-010
Dulbecco's Modified Eagle Medium (D-MEM), low glucose	500 mL	10567-014
D-MEM/F-12 (1X), liquid, 1:1 (contains GlutaMAX™-I)	500 mL	10565-018
Knockout™ D-MEM	500 mL	10829-018
Fetal Bovine Serum (FBS), ES-Cell Qualified	500 mL	16141-079
Dulbecco's Phosphate Buffered Saline (D-PBS), Calcium and Magnesium-free	500 mL 10 × 500 mL	14190-144 14190-250
MEM Non-Essential Amino Acids Solution (10 mM)	100 mL	11140-050
Knockout™ Serum Replacement	500 mL	10828-028
2-Mercaptoethanol (1,000X), liquid	50 mL	21985-023
FGF-basic, AA 10-155 Recombinant Human	50 µg	PHG0026
Recombinant Mouse Leukemia Inhibitory Factor (LIF)	10 µg 100 µg	PMC4054 PMC4051
Recombinant Human Leukemia Inhibitory Factor (LIF)	10 µg	PHC9464
L-Glutamine - 200mM (100X), liquid	100 mL	25030081
Ultrapure BSA (50 mg/mL)	50 mg	AM2616
Dispase	5 g	17105-041
StemPro® Accutase® Cell Dissociation Reagent	100 mL	A11105-01
Trypsin/EDTA Solution	100 mL	R-001-100
TrypLE™ Express Dissociation Reagent	100 mL	12604-013
Attachment Factor	100 mL	S-006-100

Continued on next page

Accessory Products, continued

Embryonic Stem Cell Lines

- The ESC lines listed below may be used with GIBCO® Mouse Embryonic Fibroblasts (Irradiated). For more information, refer to www.invitrogen.com or contact Technical Support (see page 24).

Cell Line	Quantity	Cat. no.
BG01V/hOG Cells	~2 × 10^6 cells	R7799-105
StemPro® BG01V/EG Cells	~3 × 10^6 cells	R7799-205
GIBCO® Mouse (C57) Embryonic Stem Cells	~1 × 10^6 cells	S10503-100
GIBCO® Mouse (C57) Embryonic Stem Cells with GFP	~1 × 10^6 cells	S10513-100
GIBCO® Mouse (129) Embryonic Stem Cells	~1 × 10^6 cells	S10504-100
GIBCO® Mouse (129) Embryonic Stem Cells with GFP	~1 × 10^6 cells	S10514-100

Additional Products

For more information about the following products, refer to www.invitrogen.com or contact Technical Support (see page 24).

Item	Quantity	Cat. no.
Water, distilled	500 mL	15230-162
Trypan Blue Stain	100 mL	15250-061
LIVE/DEAD® Cell Vitality Assay Kit	1000 assays	L34951
Countess™ Automated Cell Counter (includes 50 Countess™ cell counting chamber slides and 2 mL of Trypan Blue Stain)	1 unit	C10227
StemPro® EZPassage™ Disposable Stem Cell Passaging Tool	10 units	23181010

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete Technical Support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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