INSTRUCTIONS

Primary Mouse Embryonic Fibroblast Isolation Kit

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88279

Number	Description
88279	**Pierce Mouse Embryonic Fibroblast (MEF) Isolation Kit,** contains sufficient reagents to isolate MEFs from 50 embryonic mouse somatic tissues

Kit Contents:

DMEM for Primary Cell Isolation, 500mL

MEF Isolation Enzyme (with papain), lyophilized, 5 vials

Hanks' Balanced Salt Solution (HBSS without Ca^{2+}, Mg^{2+}), 500mL

Storage: Upon receipt store kit at 4°C. Product is shipped on ice packs.

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Introduction

The Thermo Scientific™ Pierce™ Mouse Embryonic Fibroblast (MEF) Isolation Kit provides a reliable and convenient method for the isolation and culture of MEFs (Figure 1). The kit consists of unique tissue-specific dissociation reagents and a validated protocol to ensure a high yield of viable MEFs when used by both experienced and non-experienced users.

MEFs isolated and cultured using the Pierce MEF Isolation Kit can be used as a cell culture model for a diverse range of studies such as gene regulation and stem cell research.[1, 2, 3, 4] MEFs are used most commonly as feeder layers to maintain mouse embryonic stem cells in an undifferentiated state. With a combination of transcription factors, MEFs can be converted to a pluripotent state or directly reprogrammed to various types of cells such as functional neurons and cardiomyocytes, which could have important implications for studies of development, neurological and cardiac disease modeling, drug discovery, and regenerative medicine.[3,5,6] The Pierce MEF Isolation Kit also can be used to isolate MEFs from different genetically altered mouse models to study growth control and DNA damage response.[7] MEFs isolated and cultured with the Pierce MEF Isolation Kit can be readily frozen for future use with minimal loss of viability.

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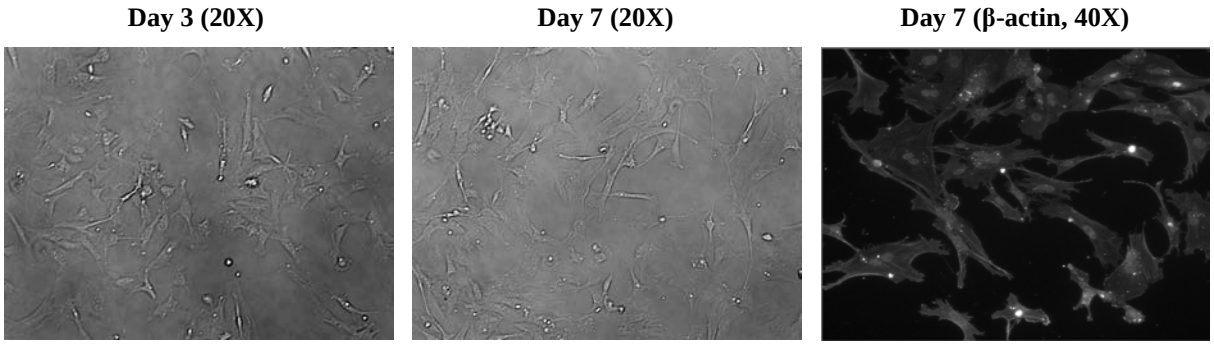

Figure 1. Examples of MEFs in culture following isolation using the Thermo Scientific Pierce Mouse Embryonic Fibroblast Isolation Kit. Phase-contrast and fluorescent images of MEFs after 3 and 7 days in culture. Cultures were plated at a density of 2 x 10^5 cells per well in a 12-well plate. For the fluorescent image (right panel), MEFs were stained with an antibody specific to β-actin. Images were taken at 20X and 40X magnifications on a Carl Zeiss microscope (AxioVision™ Rel. 4.7).

Important Product Information

- For best cell yield and viability, always isolate MEFs from freshly dissected tissues. The dissection and plating of MEFs should take no longer than two hours.
- Euthanize mice in accordance with the Guide for the Care and Use of Laboratory Animals.[8]
- Perform all tissue digestion, cell manipulations and media handling using sterile technique in a laminar flow cell culture hood to minimize contamination of the isolated MEFs.

Additional Materials Required

- Heat-inactivated fetal bovine serum (FBS) (e.g., Thermo Scientific™ Hyclone™ FBS)
- Penicillin-streptomycin (pen/strep) (e.g., Thermo Scientific™ Hyclone™ Pen/Strep Solution)
- Mouse embryonic tissue freshly dissected from mouse embryos taken from a euthanized mouse (E11-13)
- 37°C heat block or incubator
- Tissue culture incubator at 37°C with humidified, 5% CO_2 atmosphere
- Laminar flow cell culture hood
- Hemocytometer or automated cell counter for counting cells
- Trypan blue stain (e.g., Thermo Scientific™ Hyclone™ Trypan Blue)

Material Preparation

Note: After medium supplementation, medium is stable for approximately one month when stored at 4°C.

Complete DMEM for Primary Cell Isolation	Determine the amount of medium needed (see Table 2 for guidelines). In a sterile bottle, add heat-inactivated FBS (10% final concentration), and pen/strep (1% final concentration) to desired volume of DMEM for Primary Cell Isolation. Pre-warm medium to 37°C before use.

Procedure for MEF Isolation

A. Enzyme Digestion of Mouse Embryonic Tissue

Note: For the medium and buffer removal steps, it is critical to carefully remove the medium/buffer contents without disturbing the tissue. For best results, use a pipette and 1000µL tip. Do not aspirate using a vacuum flask.

Note: Equilibrate HBSS to 4°C before procedure.

1. Reconstitute the MEF Isolation Enzyme (with papain) by adding 2.5mL of HBSS to one of the vials. Mix gently for 5 minutes or until completely dissolved. Keep enzyme solution on ice.

   Note: 2.5mL of reconstituted MEF Isolation Enzyme (with papain) is sufficient for preparing 10 embryonic tissues.

   Note: Reconstituted MEF Isolation Enzyme (with papain) can be stored at -20°C for 6 months and is stable for up to two freeze-thaw cycles. The enzyme solution expires one week following preparation if stored at 4°C. Prolonged storage may result in poor performance.

2. Place freshly dissected embryonic tissue into separate 1.5mL sterile microcentrifuge tubes. Immediately add 500µL ice cold HBSS.

   Note: For the best results, use one microcentrifuge tube per one embryonic tissue.

3. Mince each embryonic tissue into 1-3mm^3 sized pieces. Wash the minced tissue with 500µL ice cold HBSS twice to remove blood from the tissue.

4. Gently remove HBSS using a pipette to the level of the tissue (450µL). Add 0.2mL reconstituted MEF Isolation Enzyme (with Papain) to each tube. Incubate tubes in a 37°C incubator for 25-30 minutes.

5. Gently remove the MEF Isolation Enzyme (with papain) solution and wash tissue twice with 500µL ice cold HBSS.

6. Add 0.5mL pre-warmed Complete DMEM for Primary Cell Isolation to each tube. Break up the tissue by pipetting up and down 15-20 times using a sterile 1.0mL pipette tip. Avoid air bubbles when pipetting.

   Note: Disrupting the tissue by pipetting improves cell yield. However, pipetting too vigorously may result in cell damage.

7. After the tissue is primarily a single-cell suspension, add 1.0mL pre-warmed Complete DMEM for Primary Cell Isolation to each tube to bring the total volume to 1.5mL.

8. Combine individual cell suspensions to determine cell concentration and cell viability.

B. Cell Yield and Viability Determination

1. Mix 25µL single cell suspension obtained in Section A, Step 8, with 25µL 0.4% trypan blue in a 1.5mL microcentrifuge tube.

2. Immediately transfer 10µL trypan blue-stained cell suspension to each of two hemocytometer counting chambers.

3. Count both the total number of cells and the number of stained (blue) cells in the hemocytometer microscopic grid.

   Cell concentration (cells/mL) = number of cells × dilution factor × 10^4

   Example: If 120 cells in a square, then $120 \times 2 \times 10^4 = 2.4 \times 10^6$ cells/mL.

   Cell yield = cell concentration × total volume of cell suspension, obtained in Section A, Step 8.

   Viability (%) = [(total cells counted – total stained cells)/total cells counted] × 100%.

   Typical cell yields and viabilities obtained are shown in Table 1.

4. If using an automated cell counter, determine cell yield and viability according to the manufacturer's instructions.

Table 1. Cell yield and viability from a typical isolation.

Cell Type	Yield (cells/mL)	Viability (Trypan Blue Exclusion)
Mouse MEF (one embryonic tissue in 1.5mL cell suspension)	9.0×10^6	90%

C. Plating and Culturing Isolated MEFs

Note: Determine the desired plating density for the cultured MEFs based on the intended downstream study. A density of 5.0×10^4 cells/cm^2 is generally recommended for culturing MEFs.

1. Pipette the appropriate cell suspension volume to each well of the culture vessel (see Table 2 for recommendations).

   Cell suspension volume/well = [required cell density × growth area (cm^2)]/cell concentration (cells/mL from Section B, Step 3)

   Example: For a 12-well Nunc™ plate, a single well is approximately 3.5cm^2. If the cell count is 2.4×10^6 cells/mL, add 83µL of cell suspension to each well.

Table 2. Recommended seeding densities for common culture vessels.*

Nunc Culture Dishes/ Chamber Slides	Well Diameter (mm)	Approximate Growth Area (cm^2)	Media Volume (mL)	Total Number of Cells Required to Seed Each Well (5×10^4 cells/cm^2)
75cm^2 flask	NA	75	15	4×10^6
35mm dish	35	9.0	2.0	5×10^5
6-well plate	35	9.6	2.0	5×10^5
12-well plate	22	3.5	0.8	2×10^5
24-well plate	16	1.8	0.5	1×10^5
48-well plate	11	1.1	0.3	5×10^4
96-well plate	4.3	0.14	0.1	1×10^4
4-well chamber slide	NA	1.8	0.5	1×10^5
8-well chamber slide	NA	0.8	0.2	4×10^4

*Additional medium may be required for long-term or less dense cultures.

2. Add Complete DMEM for Primary Cell Isolation to each well to bring the total volume to the recommended level.

   Example: For the 12-well Nunc plate example in Step 1, add 717µL Complete DMEM for Primary Cell Isolation to each well to bring the total volume to 0.8mL.

3. Incubate the dishes/chamber slides at 37°C in a 5% CO_2 incubator for 24 hours.
4. After 24 hours, replace the medium with an equivalent volume of fresh Complete DMEM for Primary Cell Isolation.
5. Place the dishes/chambers back into the 37°C, 5% CO_2 incubator.
6. Continue to feed the cultures by replacing the culture medium with fresh Complete DMEM for Primary Cell Isolation every 48-72 hours.

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	Insufficient dissociation	Use freshly reconstituted MEF Isolation Enzyme
		Confirm that the volume of MEF Isolation Enzyme used was the quantity recommended
		Follow recommended incubation time
		Check the expiration date on the product. Do not use after expiration date
	Tissue was not disrupted with pipette	Pipette tissue to aid in dissociation of MEFs
	Over-digestion	Do not exceed an enzyme incubation time of 35 minutes in Section A, Step 4
		Ensure that MEF Isolation Enzyme (with papain) is reconstituted to the recommended concentration
	Disruption by pipette was too vigorous	Do not over-pipette when disrupting digested tissue
Slow cell growth	Medium and/or supplement stored incorrectly, or expired medium and/or supplements	Confirm that the product components were stored at the proper temperature
Cell density too high or too low	Inaccurate total cell numbers seeded in the culture vessels	Use Table 2 to calculate the total cell numbers required for each type of cell culture vessel

Related Thermo Scientific Products

88290 **Mouse Embryonic Fibroblast Isolation Enzyme (with papain)**

88284 **Hanks' Balanced Salt Solution (HBSS, without Ca^{2+}, Mg^{2+}),** 500mL

88287 **DMEM for Primary Cell Isolation**

88281 **Pierce Cardiomyocyte Isolation Kit**

88288 **Cardiomyocyte Isolation Enzyme 1 (with papain)**

88289 **Cardiomyocyte Isolation Enzyme 2 (with thermolysin)**

88280 **Pierce Neuron Isolation Kit**

88285 **Neuronal Isolation Enzyme (with papain)**

88286 **Neuronal Culture Media Supplement**

88283 **Neuronal Culture Medium,** 500mL

87790 **Subcellular Protein Fractionation Kit for Tissue**

78510 **T-PER™ Tissue Protein Extraction Reagent**

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