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We use this protocol and it's working

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Isolation of mouse embryonic fibroblasts (MEFs) from mouse embryos at E12.5

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DISCLAIMER

This protocol was adapted from: Durkin ME, Qian X, Popescu NC, Lowy DR. Isolation of Mouse Embryo Fibroblasts. Bio Protoc. 2013 Sep 20;3(18):e908. doi: 10.21769/bioprotoc.908. PMID: 27376106; PMCID: PMC4928858.

ABSTRACT

Mouse embryo fibroblasts (MEFs) isolated from genetically altered mouse strains are a powerful resource for investigating the consequences of defined genetic mutations at the cellular level. Here we describe a protocol for the isolation of MEFs from mouse embryos collected at E12.5. MEFs obtained by this procedure are suitable for use in biochemical assays and for further genetic manipulation (e.g. viral transduction).
This protocol was adapted from: Durkin ME, Qian X, Popescu NC, Lowy DR. Isolation of Mouse Embryo Fibroblasts. Bio Protoc. 2013 Sep 20;3(18):e908. doi: 10.21769/bioprotoc.908. PMID: 27376106; PMCID: PMC4928858.

GUIDELINES

Perform procedure in a tissue culture laminar flow hood using aseptic technique.
CRITICAL All experiments must be conducted in accordance with the relevant institutional and governmental guidelines and regulations.

MATERIALS

Materials and Reagents:

- Mouse embryos at E12.5 (intact uterus dissected and transferred to ice cold PBS).

The morning the copulation plug is found is designated as day 0.5. Harvest embryos from female mice 12 days after the appearance of the copulation plug for mouse embryos at E12.5 (or 12.5 dpc: days post coitum).

- 70% (v/v) ethanol
- Phosphate buffered saline (PBS), without Ca^{2+} and Mg^{2+} (Gibco™, catalog number: 14190169, or equivalent), ice cold
- 0.05% trypsin-EDTA (Gibco™, catalog number: 25300054, or equivalent)
- Dulbecco's Modified Eagle's Medium (DMEM), High Glucose, no glutamine (Gibco™, catalog number: 11960044, or equivalent)
- Fetal bovine serum (FBS) (Sigma, F7524, or equivalent)
- L-Glutamine (200mM) (Gibco™, catalog number: 25030024, or equivalent)
- Penicillin-Streptomycin 10.000U/mL (Gibco™, catalog number: 15140122, or equivalent)
- Sodium Pyruvate (100mM) (Gibco™, 11360039, or equivalent)
- Non-Essential Amino Acids Solution 100X (Gibco™, 11140035, or equivalent)
- MEF culture medium: DMEM supplemented with 10% (v/v) FBS, 2 mM L-glutamine, Penicillin-Streptomycin 100U/mL, 1 mM Sodium Pyruvate, 1X NEAA solution

Equipment:

- Sterile fine scissors
- Sterile fine forceps
- Sterile scalpel blades
- Petri dishes (100 mm) (BD Biosciences, Falcon®, catalog number: 351029, or equivalent)
- Sterile plastic serological pipettes
- Sterile conical centrifuge tubes (15 ml) (BD Biosciences, Falcon®, catalog number: 352095, or equivalent)
- Humidified tissue culture incubator, set at 37°C, 5% (v/v) CO_2
- Tissue culture laminar flow hood
- Inverted microscope

1 Transfer the uterus containing the embryos to a dish with ice cold PBS.

2 Separate the embryos by cutting through the uterus in the region between each embryo with scissors.

3 Transfer each embryo to a new dish with ice cold PBS.

4 Remove the placenta and other maternal tissues by gently pulling them off using forceps. If the embryo is still in the yolk sac, gently pull the sac off with forceps.
Note: Clean scissors and forceps with 70% ethanol between each embryo.

5 Pick up the embryo with forceps and transfer to a fresh dish.
Note: Perform steps 6-10 as quickly as possible to avoid the embryo drying out.

6 Cut off the embryo head (eyes and above).
Note: The head can be used for DNA isolation for genotyping.

7 Remove the red parts (heart and liver) from the embryo by gently scraping with forceps.

8 Place the rest of the embryo in a new dish.

9 Using a scalpel blade, finely chop the embryo into pieces of 1–2 mm.
Note: Use a new scalpel for each embryo.

10 Add 0.05% trypsin-EDTA (around 7.5 mL), ensuring the embryo pieces are completely covered.

11 Place the dish in the tissue culture incubator for 10-15 min.

12 After 10-15 min incubation with 0.05% trypsin-EDTA, check the plate under the microscope. If a lot of cells are floating, proceed to step 13, otherwise incubate for further 5-10 min at 37°C.

13 Add 7.5 ml MEF culture medium to inactivate the trypsin.

14 Gently pipet up and down several times.

15 Transfer the cell suspension to a 15 ml conical centrifuge tube.

16 Spin tube at 180 x g for 5 min.

17 Carefully aspirate the supernatant without disturbing the pellet.

18 Re-suspend the cell pellet in 10 ml of media by gently pipetting up and down several times.

19 Transfer the cell suspension to a fresh dish.

20 Transfer the dishes to the incubator.

21 The following day, replace the old medium with fresh culture medium to get rid of dead cells and debris.

22 Split the cells when they reach confluency (this is passage number 1). Alternatively, cells can be frozen down (freezing medium: 90% v/v MEF culture medium, 10% v/v DMSO).

23 *MEF obtained by this procedure can be immortalised either by serial passaging, or by SV40-mediated immortalisation.*