

Mouse Adipose-Derived Mesenchymal Stem Cell Isolation and Culture Kit

Cat. No.: P-CA-720

Size: 3Tests/10Tests

Background

The Mouse Adipose-Derived Mesenchymal Stem Cell Isolation and Culture Kit is specifically developed for the extraction of primary mouse adipose-derived mesenchymal stem cells. Verified through standardized procedures, each Test of this kit supports the acquisition of one flask of cells (6cm culture dish), with a cell count exceeding 1×10^6 cells. The cells were subcultured at a ratio of 1:2 and could be propagated for 2–3 passages. Immunofluorescence identification confirmed that the cell purity (positive rates for CD73, CD90, CD105, CD29, and CD44) was > 90%.

Scope of Application

This product is suitable for extracting The Mouse Adipose-Derived Mesenchymal Stem Cells from 20–40-day-old mice of various strains, such as KM and C57. Following standard tissue isolation and plating procedures, it can yield $>1 \times 10^6$ cells.

Note: White adipose tissue was harvested from the inguinal region of six mice (alternatively, adipose tissue surrounding the testis or uterus may be collected). Tissues from different anatomical sites should not be pooled for extraction. The yield obtained from this procedure is sufficient for one 6 cm cell culture dish. The exact number of mice required may vary depending on the amount of adipose tissue recovered during harvesting.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells	3Tests (250 mL) 10Tests (500 mL×2)	Pale Yellow Clear Liquid	2–8°C, 1 year
Specialized Digestive Solution for Mouse Adipose-Derived Mesenchymal Stem Cells	3Tests (15 mL) 10Tests (50 mL)	Pale Yellow Clear Liquid	–5~–20°C, 1 year
Basic Culture Medium for Mouse Adipose-Derived Mesenchymal Stem Cells	3Tests (100 mL) 10Tests (250 mL)	Red Clear Liquid	2–8°C, 1 year
Supplement for Mouse Adipose-Derived Mesenchymal Stem Cells	3Tests (10 mL) 10Tests (25 mL)	Yellow Clear Liquid	–5~–20°C, 1 year
70 µm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	N/A	Room temperature, 3 years
100 µm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	N/A	Room temperature, 3 years

Note: All components should be stored according to the temperature indicated on the labels of the reagent tubes. The reagents stored at –5 to –20°C (such as Supplement for Mouse Adipose-Derived Mesenchymal Stem Cells) can be preserved at 4°C for 30 days after thawing. For long-term storage, aliquot them into single-use portions and store at –20°C. Thaw again at 4°C before use to avoid repeated freeze-thaw cycles.

Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. Prior to formal experiments, it is recommended to conduct anatomical simulation training using 1-

2 normal mice to familiarize yourself with operational procedures and improve tissue isolation efficiency.

3. Reagent preparation or aliquoting must strictly adhere to aseptic techniques. After dispensing, seal the containers immediately with a sealing film, use them promptly to avoid repeated freeze-thaw cycles or contamination.

Operational Procedures

1. Pre-experiment Preparations

- 1) Self-supplied Reagents and Consumables: Phosphate-Buffered Saline (PBS), surgical instruments (At least 4 pairs of ophthalmic scissors, 1 pair of straight forceps, 2 pairs of curved forceps, 1 pair of straight microforceps and 1 pair of curved microforceps.), 6 cm/10 cm culture dishes, T25 culture flasks, dissection board (foam board can substitute) and multiple 2 mL/15 mL/50 mL centrifuge tubes.
- 2) Reagent Thawing and Rewarming:
 - ① Supplement for Mouse Adipose-Derived Mesenchymal Stem Cells & Specialized Digestive Solution for Mouse Adipose-Derived Mesenchymal Stem Cells: Thaw at 2-8°C and equilibrate to room temperature.
 - ② Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells & Basic Culture Medium for Mouse Adipose-Derived Mesenchymal Stem Cells: Equilibrate to room temperature.
- 3) Preparation of Complete Culture Medium: Add 10 mL of Supplement for Mouse Adipose-Derived Mesenchymal Stem Cells to 100 mL of Basic Culture Medium for Mouse Adipose-Derived Mesenchymal Stem Cells, then mix thoroughly.

Note: Storage conditions for complete culture medium: 2-8°C, valid for 3 months. When preparing the complete culture medium, it can be prepared according to the usage amount. Remaining supplement should be aliquoted proportionally and stored at -5~-20°C to avoid repeated freeze-thaw cycles.

2. Dissection Procedures

- 1) Animal Euthanasia and Disinfection Protocol: Perform euthanasia via pentobarbital sodium overdose injection or cervical dislocation, then immerse the carcass in 75% medical-grade ethanol for 5 minutes for disinfection. After disinfection, transfer the animal to a clean bench for subsequent procedures.
- 2) Dissection and Tissue Harvesting Steps:
 - ① Preparation: Arrange sterilized scissors and forceps in pairs in order of use from left to right on two sterilized EP tube racks (Ophthalmic Scissors 1 and Straight Forceps 1, Ophthalmic Scissors 2 and Curved Forceps 2, Ophthalmic Scissors 3 and Curved Forceps 3).

Note: The distal third of the instruments should extend beyond the rack to avoid contamination. After each use, return tools to their original positions and make sure they don't touch each other to prevent cross-contact.
 - ② Mouse Fixation: Secure the mouse in a supine position on the clean bench using needles, preparing for tissue harvesting.
 - ③ Tissue Harvesting Procedure:
 - a. Using straight forceps 1 to grasp and secure the skin over the thoracoabdominal region. Use ophthalmic scissors 1 to make a longitudinal incision along the midline of the thoracoabdominal skin. Use straight forceps 1 to lift the skin at the incision site, and then use ophthalmic scissors 1 to make transverse incisions toward the four limbs to separate the skin from the underlying tissue. Carefully detach and scrape the skin away from the subcutaneous adhesions using ophthalmic scissors 1, taking care not to puncture the

peritoneum. Assisted by straight forceps 1 peel the skin away and retract it laterally to expose the superficial abdominal muscular layer.

Note: Cut the skin to expose the entire abdomen. Do not puncture the peritoneum. Take care to pull the fur away from the dissection area to prevent contamination.

- b. Fix and clamp the peritoneum with Curved Forceps 2, and cut open the peritoneum with Ophthalmic Scissors 2 to fully expose the adipose tissue surrounding the uterus. Hold the adipose tissue with curved forceps 3, and dissect and harvest the intact adipose tissue with ophthalmic scissors 3. Place the adipose tissue into a petri dish containing 10 mL of Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells (Figure 1).

Note: Take care not to cut or puncture the intestinal tissue, as this may increase the risk of contamination. During the entire harvesting procedure, only the first pair of scissors and forceps should be used to contact the external skin of the mouse. All other instruments must be kept away from the skin and hair. Should any inadvertent contact occur, the instrument involved must be replaced with a sterile one to avoid contamination.

3. Tissue Processing and Digestion

1) Tissue Processing

- ① Put Straight Microforceps, Curved Microforceps and Ophthalmic Scissors 4 on the EP tube rack within the clean bench, ensuring the distal third of each tool suspended.
- ② Use the new set of microforceps to process the adipose tissue. Hold the straight-shaped microforceps 4 with the left hand to fix the adipose tissue, and use the curved-shaped microforceps 4 in the right hand to carefully separate the fascia and blood vessels from the adipose tissue. (Fig. 2). After cleaning, transfer the tissue into a culture dish containing 10 mL of Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells.
- ③ Transfer the processed adipose tissue to a 2 mL centrifuge tube. Add 0.5 mL of Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells to the tube, and use ophthalmic scissors 4 to rapidly mince the adipose tissue into fragments of approximately 1 mm³ (criteria for adequate mincing: the tissue suspension can be aspirated smoothly and without resistance using a 5 mL pipette). Transfer the minced tissue to a 15 mL centrifuge tube using a 5 mL pipette or a Pasteur pipette, and add 10 mL of Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells to resuspend the pellet. Place the tube in a benchtop centrifuge (room temperature) and centrifuge at 1,200 rpm for 1 min. Carefully aspirate the floating adipose tissue from the uppermost layer, taking care to avoid drawing up the liquid, and transfer it to a new 15 mL centrifuge tube.

2) Tissue Digestion

- ① Add 5 mL of Specialized Digestive Solution for Mouse Adipose-Derived Mesenchymal Stem Cells to the centrifuge tube, and mix thoroughly by pipetting. Place the tube in a 37°C water bath shaker at 150 rpm for 1 hour of digestion.
- ② After digestion is complete, remove the tube from the shaker. Using a 5 mL pipette, pipette the tissue suspension vigorously for 30 times. Add 5 mL of Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells to the tube to dilute the digestion solution. Pre-wet both sides of the cell strainers with Specialized Washing Solution for Mouse Adipose-Derived Mesenchymal Stem Cells, and sequentially filter the suspension through 100 µm and 70 µm cell strainers (criteria for acceptable filtration: no visible gelatinous material and smooth flow through the strainer). Collect the filtrate into a 15 mL centrifuge tube.

3) Cell Isolation

- ① Centrifuge the 15 mL centrifuge tube at 1,600 rpm for 10 min. Discard the supernatant and retain the pellet.
- ② Add 10 mL of PBS to the 15 mL centrifuge tube to resuspend the pellet, and centrifuge at 1,600 rpm for 10 min. Discard the supernatant and retain the pellet.

4. Cell Culture and Subculture

- 1) Cell Seeding: Take out the culture dish or T25 culture flask for the next experiment, and resuspend the cell pellet in the centrifuge tube with 5 mL of Complete Culture Medium of Mouse Adipose-Derived Mesenchymal Stem Cells, then inoculate into the culture dish. The cells were cultured in a incubator at 37°C, 5% CO₂.
- 2) Medium Renewal: Perform the first medium change at 48 hours. And subsequent medium replacements are performed on the third day, the fifth day, and then every 2-3 days. After inoculation for about 3 days, cell confluence will reach 80–90%.
- 3) Cell Subculture: When the cell confluence reaches 80–90%, the cells are ready for passage. First, aspirate the culture medium from the 6 cm cell culture dish and wash the cells once with 2–3 mL of PBS. Add 1 mL of 0.25% Trypsin–EDTA digestion solution to the 6 cm dish and gently rotate the dish to ensure the solution completely covers the bottom. Aspirate the excess trypsin digestion solution and incubate at 37°C for 1–3 min. Observe under an inverted microscope. When the cells retract and become rounded, gently tap the culture plate until most of the cells detach, then add 5 mL Complete Medium for Mouse Adipose-Derived Mesenchymal Stem Cells to terminate digestion. Gently pipette with a 5 mL pipette or a Pasteur pipette to mix thoroughly. According to the subculture ratio or experimental requirements, seed the cells into new culture vessels and incubate statically in a 37°C, 5% CO₂ constant-temperature incubator.

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	Insufficient tissue yield during harvesting	If the experimental animals are not sufficiently obese and yield insufficient adipose tissue, the number of mice may be appropriately increased, or obese mice may be used instead.
	Insufficient tissue digestion.	Mince adipose tissue thoroughly; store Digestion Solution properly to avoid enzyme inactivation.
The cell adheres slowly	Improper preparation of culture medium	Prepare complete culture medium with accurate ratios and avoid repeated freeze-thaw cycles
		Use the complete culture medium within its validity period and avoid preparing it for more than three months
	The age of the mouse is inappropriate	Use mouse aged 20-40 days postnatally. The different ages of mice may lead to slow cell adhesion and poor cell condition. It is recommended to perform centrifugal fluid replacement and continue the culture for several days for observation
Low cell purity	Incomplete removal of vascular tissue.	Ensure complete removal of vascular tissue.

Anatomy Images for Reference

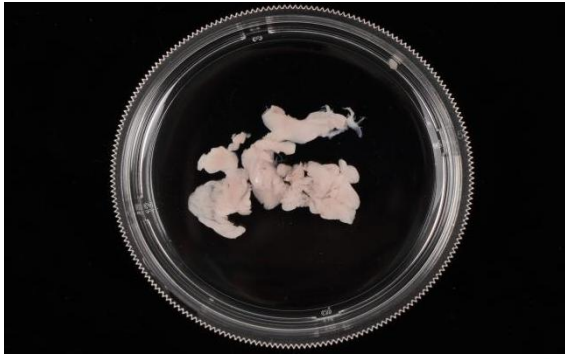


Figure 1. Obtain intact adipose tissue.

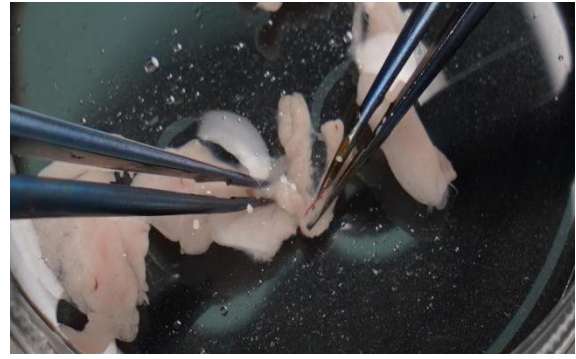


Figure 2. Carefully remove fascia and blood vessels from adipose tissue.



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