Mouse Tail Tendon Stem Cell Isolation and Culture Kit

Cat. No.: P-CA-719

Size: 3Tests / 10Tests

Background

The Mouse Tail Tendon Stem Cell Isolation and Culture Kit is specifically developed for the extraction of primary mouse tail tendon stem cells. Verified through standardized procedures, each Test of this kit supports the acquisition of one flask of cells (6 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 CD90 and CD44) was > 90%.

Scope of Application

This product is suitable for extracting the mouse tail tendon stem cells from 14-30-day-old mice of various strains, such as KM and C57. Following standard tissue isolation and plating procedures, it can yield >1×10^6 cells.

Note: Tendon tissue was harvested from the tails of four mice. The yield obtained from this procedure is sufficient for one flask of cells (T-25 culture flask). The exact number of mice required may vary depending on the amount of tendon tissue obtained.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution for Mouse Tail Tendon Stem Cells	3Tests (250 mL) 10Tests (500 mL×2)	Pale Yellow Clear Liquid	2-8°C, 1 year
Specialized Digestive Solution for Mouse Tail Tendon Stem Cells	3Tests (15 mL) 10Tests (50 mL)	Pale Yellow Clear Liquid	-5~-20°C, 1 year
Basic Culture Medium for Mouse Tail Tendon Stem Cells	3Tests (100 mL) 10Tests (250 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement for Mouse Tail Tendon Stem Cells	3Tests (10 mL) 10Tests (25 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
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 Tail Tendon 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 3 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 Tail Tendon Stem Cells & Specialized Digestive Solution for Mouse Tail Tendon Stem Cells: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution for Mouse Tail Tendon Stem Cells & Basic Culture Medium for Mouse Tail Tendon Stem Cells: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 5 mL of Supplement for Mouse Tail Tendon Stem Cells to 50 mL of Basic Culture Medium for Mouse Tail Tendon 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 -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 Straight Forceps 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-contamination.
   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. Use straight forceps 1 to grasp and secure the mouse tail, and use ophthalmic scissors 1 to cut through the upper-middle portion of the tail. Place the severed tail tissue into a culture dish (Figure 1).
      b. Use straight forceps 2 to grasp and secure the tail, and use ophthalmic scissors 2 to make a small incision (Figure 2). Switch to straight forceps 3 to grasp the incision edge, avoiding the fur as much as possible. Use curved forceps 3 to grasp the skin and peel it off in one motion, then discard it (Figure 3). Transfer the denuded tail tissue into a culture dish containing approximately 10 mL of Wash Solution for Mouse Tail Tendon Stem Cells using straight forceps 3 (Figure 4). To avoid contamination, the tissue may be rinsed multiple times by transferring it to fresh culture dishes as needed.

3. Tissue Processing and Digestion

1) Tissue Processing

① Put Straight Microforceps, Curved Microforceps and Ophthalmic Scissors 3 on the EP tube rack within the clean bench, ensuring the distal third of each tool is suspended.

② Use the new set of microforceps to process the tail tissue. Secure the tail tissue with straight microforceps 1 in the left hand, and carefully pull out the tail tendon tissue with curved microforceps 1 in the right hand (Figure 5). Place the isolated tendon tissue into a culture dish containing approximately 10 mL of Wash Solution for Mouse Tail Tendon Stem Cells (Figure 6). In the dish, remove the fascia and other connective tissue to obtain clean tail tendon tissue, and rinse it once by transferring it to another culture dish containing 10 mL of Wash Solution for Mouse Tail Tendon Stem Cells (Figure 7).

③ Transfer the processed tail tendon tissue to a culture dish, add 1 mL of Wash Solution for Mouse Tail Tendon Stem Cells, and mince the tissue with ophthalmic scissors 3 (Figure 8). If the tissue fragments adhere together, use straight and curved microforceps to tease them apart and continue mincing until the tail tendon tissue is cut into 1 mm^3 fragments (Figure 9). Transfer the minced tissue to a 15 mL centrifuge tube using a 1 mL pipette or a Pasteur pipette (to prevent tissue from sticking to the pipette tip, pre-rinse the tip with Wash Solution for Mouse Tail Tendon Stem Cells before aspirating the tissue). Add 10 mL of Wash Solution for Mouse Tail Tendon Stem Cells to wash the dish and transfer the remaining tissue to the centrifuge tube. Place the tube in a benchtop centrifuge (room temperature) and centrifuge at 1,200 rpm for 1 min. Aspirate and discard the supernatant, and retain the pellet.

2) Tissue Digestion

① Add 5 mL of Specialized Digestive Solution for Mouse Tail Tendon Stem Cells to the centrifuge tube, and mix thoroughly by pipetting. Place the tube in a 4°C refrigerator for digestion for 16–18 hours.

② After digestion is complete, remove the centrifuge tube from the 4°C refrigerator. Using a 5 mL pipette, pipette the tissue suspension vigorously 30 times. Add 5 mL of Specialized Washing Solution for Mouse Tail Tendon Stem Cells to the tube to dilute the digestion solution.

3) Cell Isolation

① Place a 100 μm cell strainer on the opening of a new 50 mL centrifuge tube, and pre-wet the strainer with 2–3 mL of Specialized Washing Solution for Mouse Tail Tendon Stem Cells.

② Carefully aspirate the tail tendon stem cell suspension from the previous step using a 5 mL pipette or a Pasteur pipette, and filter it through the 100 μm cell strainer. After filtration, gently add 2–3 mL of Specialized Washing Solution for Mouse Tail Tendon Stem Cells onto the strainer using a clean pipette tip to collect the remaining cell suspension retained on the strainer. Collect the filtrate into a 50 mL centrifuge tube. Centrifuge the 50 mL tube at 1,500 rpm for 5 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 Tail Tendon Stem Cells, then inoculate into the culture dish. The cells were cultured in a incubator at 37°C, 5% CO_2.

2) Medium Renewal: Perform the first medium change at 72 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 T25 culture flask and wash the cells once with 2–3 mL of PBS. Add 1 mL of 0.25% Trypsin–EDTA digestion solution to the T25 culture flask 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 Tail Tendon 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% CO_2 constant-temperature incubator.

Troubleshooting

Problem	Possible Cause	Solution
Low cell count	Insufficient tissue digestion.	Ensure tissue is thoroughly minced to prevent tissue from sticking together.
The cells 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 mice aged 14-30 days postnatally. If cells do not adhere to the wall due to different days of age, centrifuge and change the culture for several days
Low cell purity	Muscle tissue not completely removed	Ensure complete removal of other tissues and obtain pure tail tendon tissue

Anatomy Images for Reference



Figure 1. Place the tail tissue into a petri dish.



Figure 2. Cut out a gap

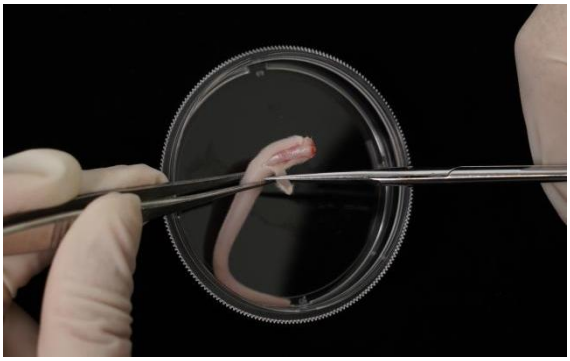


Figure 2. Cut out a gap



Figure 3. Pinch the fur and tear it off smoothly, then discard it.



Figure 4. Place the peeled tail tissue into a culture dish.

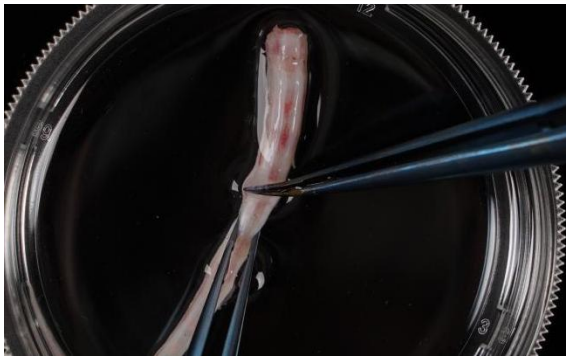


Figure5. Carefully lift and retract the tail tendon tissue.

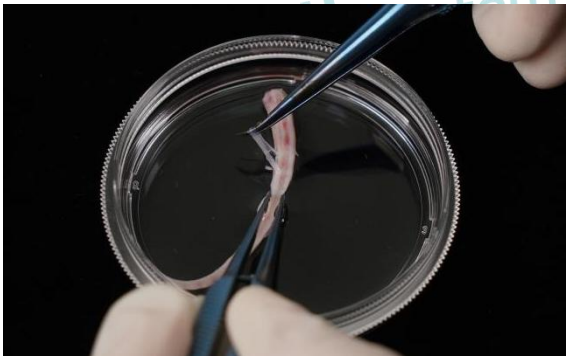


Figure5. Carefully lift and retract the tail tendon tissue.



Figure 6. Place in a petri dish.



Figure 7. Cleaned tail tendon tissue



Figure 8. Shred the tail tendon tissue.

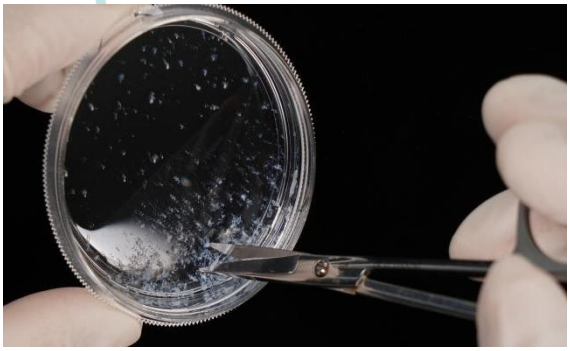


Figure 8. Shred the tail tendon tissue.

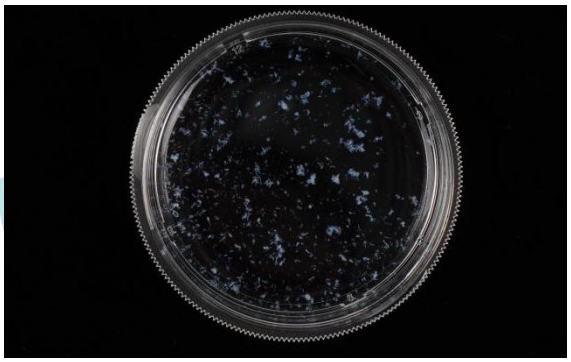


Figure 9. The tail tendon tissue was cut into fragments of 1 mm³ each.