Rat Neural Stem Cell Isolation and Culture Kit

Cat. No.: P-CA-603

Size: 3Tests/10Tests

Background

The Rat Neural Stem Cell Isolation and Culture Kit is specifically developed to extract primary Rat Neural Stem Cells. As validated, standard operation using this kit enables the acquisition of one flask of cells (T-25 culture flask) per 1 Test, with a cell count exceeding 1×10^6 cells. When passaged at a 1:2 ratio, the cells can undergo 2-3 passages. Through immunofluorescence analysis, the cell purity (Nestin-positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for isolating Neural Stem Cells from 1-2 day old rats of various strains, such as Wistar and SD. Through processes of tissue isolation, enzymatic digestion, and 48-hour planting purification, a yield of $>1\times10^6$ cells can be obtained.

Note: The extraction of intact brain tissue from 4 rats (8 intact cerebral hemispheres) is required to yield sufficient cells for one T-25 flask. The exact number of rats required may vary depending on the size and quantity of brain tissue harvested during the procedure.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Rat Neural Stem Cells	3Tests (250 mL) 10Tests (500 mL×2)	Faint Yellow Clear Liquid	2-8℃, 1 year
Specialized Digestive Solution For Rat Neural Stem Cells	3Tests (24 mL) 10Tests (80 mL)	Colorless Clear Liquid	-5~-20℃, 1 year
Basic Culture Medium For Rat Neural Stem Cells	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8℃, 1 year
Supplement For Rat Neural Stem Cells	3Tests (5 mL) 10Tests (10 mL)	Yellow Clear Liquid	-5~-20℃, 1 year
Digestion Termination Solution For Rat Neural Stem Cells	3Tests (75 mL) 10Tests (250 mL)	Red Clear Liquid	2-8℃, 1 year
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Orange	Room temperature, 3 years
100 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Green	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~-20℃ (such as Specialized Digestive Solution for Rat Neural Stem Cells) can be preserved at 4℃ for 30 days after thawing. For long-term storage, aliquot them into single-use portions and freeze at -5~-20℃. Thaw again before use to avoid repeated freeze-thaw cycles.

Note

1. Prior to formal experiments, it is recommended to conduct anatomical simulation training using 1-2 normal rats to familiarize operators with procedural workflows and improve tissue dissociation efficiency.
2. During the entire tissue dissociation process, place the small dish containing the tissue on an ice tray/ice box

(2-8℃) to maintain hypothermic conditions. Critical precautions: Monitor temperature rigorously to prevent ice crystal formation in tissues/liquids.

3. Reagent preparation or dispensing must strictly adhere to aseptic technique protocols. After dispensing, seal the containers immediately with a sealing film, and use them promptly to avoid repeated freeze-thaw cycles or contamination.
4. It is recommended to culture cells using non-TC-treated T25 flasks or 6 cm dishes. If unavailable, TC-treated T25 flasks can be substituted. If cell adhesion occurs (a normal phenomenon), gently tap the flask to detach the adhered cells and proceed with trypsinization.

Operational Procedures

1. Pre-experiment Preparation

1) Self-supplied Reagents and Consumables: two Eppendorf (EP) tube racks; one ice tray/ice plate; 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 micro straight forceps; 1 pair of micro curved forceps); 6 cm/10 cm culture dishes; T25 culture flask; dissection board (foam board substitute): assorted 2 mL/15 mL/50 mL centrifuge tubes; sterile filter paper (optional); Accutase Cell Digestive Solution.
2) Reagent Thawing and Rewarming:
   ① Specialized Digestive Solution For Rat Neural Stem Cells; Supplement For Rat Neural Stem Cells: Thaw at 4℃ and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Neural Stem Cells; Basic Culture Medium For Rat Neural Stem Cells; Digestion Termination Solution For Rat Neural Stem Cells: Equilibrate to room temperature.
3) Preparation of complete culture medium: Add 2.5 mL of Supplement For Rat Neural Stem Cells into 50 mL of Basic Culture Medium For Rat Neural Stem Cells, mix thoroughly.

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

2. Dissection Protocol:

1) Animal Disinfection and Processing: After euthanizing the animal via decapitation, spray the carcass with 75% medical alcohol (ethanol disinfectant) for disinfection. Upon completion of disinfection, transfer the animal head to a clean bench for subsequent procedures.
2) Dissection and Tissue Harvesting Steps:
   ① Preparation: Arrange sterilized scissors and forceps in pairs (ophthalmic scissors and straight/curved forceps) 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 Rat fixation: Secure the rat in a prone position within the clean bench using needles for stabilization during tissue harvesting.
   ③ Tissue Sampling:
      a. Place the severed head in a new sterile culture dish. Add 10 mL of Specialized Washing Solution For Rat Neural Stem Cells to another clean dish and place it on an ice tray.
      b. Stabilize the rat's mouth vertically with Straight Forceps 1. Use Ophthalmic Scissors 1 to cut the skin along the midline of the skull from the head to the nose, then extend the incision downward bilaterally to the mandible. Reflect the skin laterally to fully expose the skull.

      Note: Ensure the eyes are exposed during this step.

      c. Skull Opening: Maintain fixation with Straight Forceps 1. Switch to Ophthalmic Scissors 2, insert

the lower blade into the cervical spine and the upper blade above the skull. Cut along the cranial midline.

Note: Do not insert the scissors too deep into the inside of the head. Cut lightly upward and forward to avoid cutting the brain tissue under the skull.

d. Skull Removal: Fix the head with Straight Forceps 1. Position Ophthalmic Scissors 2 parallel to the rat's body. Insert the blade on one side of the skull base along the cut surface of the neck. Place one side of the blade outside the skull to cut off the connection between the skull base and both sides. Use curved forceps 2 to tear the skull open to both sides.

Note: Use Curved Forceps 2 to clamp only the skull, and avoid clamping the brain tissue to prevent it from being crushed or contaminated.

e. Brain Extraction: Fix the head with Straight Forceps 1. Gently lift the brain using Curved Forceps 3 (handle with care to prevent fragmentation). Separate the intact brain and transfer it to the dish containing 10 mL of Specialized Washing Solution For Rat Neural Stem Cells (Figure 1). Maintain the dish on ice to preserve a low-temperature environment.

Note: Only the first set of instruments may contact external skin; Other instruments are strictly prohibited from touching external skin and hair. If they do, sterile instruments must be replaced to prevent contamination. Periodically agitate the dish to prevent tissue freezing during prolonged procedures.

3. Tissue Processing and Digestion

1) Tissue Processing

① Put straight micro forceps and curved micro forceps on the EP tube rack within the biosafety cabinet, ensuring their tips remain suspended.

② Brain tissue dissection: Tissue dissection with the new micro forceps set. Fixate the brain tissue with straight micro forceps in left hand. With the curved-tip forceps in the right hand, clamp along the three black solid lines (Figure 2) to separate the left/right cerebral hemispheres and cerebellum.

③ Cortical tissue purification: Flip the cerebral hemispheres (Figure 3), identify the boundary between the cortex and medulla. Remove the medulla (Figure 4), retain the cortical tissue, and transfer it to a Petri dish containing 10 mL of Specialized Washing Solution For Rat Neural Stem Cells.

④ Meninges Removal (Two approaches):

Option 1:

Flip the hemispheres again, position the meningeal surface upward (Figure 5). Use micro curved-tip forceps to tear the meninges (Figure 6) partially. Grasp the torn meninges with the forceps, stabilize the tissue with the left hand, and gently pull to strip off the meninges (Figure 7). Retain the purified cortical tissue.

Note: Select regions with abundant cortical blood vessels for easier meningeal lifting. Complete removal of meninges is critical to avoid contamination.

Option 2:

Place sterile filter paper in a petri dish. Transfer cortical tissue onto the filter paper. Roll the tissue 1–2 times using micro forceps. The red meningeal layer will detach, leaving purified cortical tissue.

⑤ Tissue fragmentation: Transfer the processed cortical tissue into two or three 2 mL microcentrifuge tubes. Add 0.5 mL Specialized Washing Solution For Rat Neural Stem Cells to each tube. Rapidly mince the tissue into about 1 mm^3 fragments using Ophthalmic Scissors 3 (about 200 cuts required). Transfer the fragments to a 15 mL centrifuge tube using a 5 mL pipette or Pasteur Pipette. Resuspend the pellet in 10 mL washing solution. Centrifuge at 1200 rpm for 1 min (room temperature). Discard supernatant, retain pellet.

2) Tissue Digestion

① Add 8 mL of Specialized Digestive Solution For Rat Neural Stem Cells to the centrifuge tube. Mix gently,

seal the tube opening with parafilm, and incubate at 37℃, 5% CO_2 for 5 minutes.

② Transfer the tube to a 37℃ water bath shaker and digest at 150 rpm for 15 minutes.

③ Termination of digestion: Prepare a new 50 mL centrifuge tube containing 20 mL Digestion Termination Solution For Rat Neural Stem Cells. After digestion, gently pipette the tissue-digestive solution mixture 10 times using a 5 mL pipette or Pasteur pipette to homogenize. Transfer the suspension to the termination solution-containing tube. Pipette 20 times until no visible tissue clumps remain.

Note: The tissue fluid is very viscous when it is blown for the first time and is difficult to aspirate. The viscosity will decrease after several blows.

④ Cell Filter Preparation: Place 100 μm Cell Filter and 70 μm Cell Filter onto two new 50 mL centrifuge tubes. Rinse both filters with Specialized Washing Solution For Rat Neural Stem Cells.

⑤ Filtration and Collection: Filter the suspension sequentially through the 100 μm and 70 μm filters. After filtration, slowly add 3-5 mL Specialized Washing Solution For Rat Neural Stem Cells to the filter surface to collect residual cells.

Note: Ensure smooth filtration; if clogged, tilt the filter slightly to improve flow. Avoid excessive colloidal substance retention on the filter.

⑥ Centrifugation and Resuspension: Centrifuge the filtered suspension at 410× g for 5 minutes. Discard supernatant and resuspend the pellet in 10 mL Specialized Washing Solution. Transfer to a 15 mL tube, centrifuge at 230× g for 5 minutes, discard supernatant, and retain the pellet for downstream applications.

4. Cell Culture

1) Cell seeding: Take out the T25 cell culture flask. Resuspend the cell pellet in 5 mL of Complete Medium For Rat Neural Stem Cells and transfer the cell suspension into the non-TC-treated T25 flask. Incubate at 37℃ with 5% CO_2 for static culture for 48 h.

2) Medium replacement: Exchange the culture medium every 2-3 days. Aspirate the supernatant from the culture flask into a 15 mL centrifuge tube, centrifuge at 1200 rpm for 3 min, discard the supernatant, resuspend the cell pellet in Complete Medium For Rat Neural Stem Cells, and transfer the suspension back to the culture flask.

3) Cell passaging protocol: Passage should be initiated when the center of the neurospheres begins to darken. The detailed procedure is as follows:

① Transfer the cell suspension from the T25 flask to a 15 mL sterile centrifuge tube. Centrifuge at 1200 rpm for 3 min, then discard the supernatant.

② Add 2 mL PBS to resuspend the precipitate, followed by centrifugation at 1200 rpm for 3 minutes. Discard the supernatant and retain the cell pellet.

③ Add 1 mL Accutase Cell Digestive Solution to the pellet, gently resuspend using a pipette tip, and incubate at 4℃ for 15-20 minutes.

④ Add 5 mL PBS to neutralize the digestion, then gently pipette to dissociate the neurospheres into a single-cell suspension.

⑤ Centrifuge the suspension at 1200 rpm for 5 minutes and discard the supernatant. Collect the cell pellet.

⑥ Resuspend the pellet in 5 mL Complete Medium For Rat Neural Stem Cells and seed cells at the desired ratio. Adjust the total volume to 5 mL with complete medium. Incubate in a 37℃, 5% CO_2 humidified incubator.

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	Insufficient dissociation	Check the storage conditions of digestion solution to ensure it has not been stored at 4℃ for more than 30 days.
		Ensure the tissue quantity matches the kit requirements.
		Ensure tissue is gently pipetted thoroughly
	Over-digestion	Strictly control the digestion time.
	Tissue with inadequate freshness	Accelerate tissue collection speed to prevent prolonged storage.
		Ensure that post-sampling tissues are consistently maintained under low-temperature conditions.
Slow cell growth	Improper preparation of culture medium	Prepare complete culture medium with accurate ratios and avoid repeated freeze-thaw cycles
		Use complete culture medium within its validity period and avoid preparation for longer than three months
	Over-aged rats	Use rats at 1-2 days postnatal age to avoid slower proliferation and reduced passage numbers in older specimens
	Improper passaging ratio	When passaging at 1:2 ratio, calculate based on vessel surface area to maintain proper cell seeding density
	Over-passaged	Limit cell passage to 2-3 times to prevent proliferation slowdown
	Excessive use of pipette to blow cells	Gently blow the cells

Anatomy Images for Reference



Figure 1 Separated brain

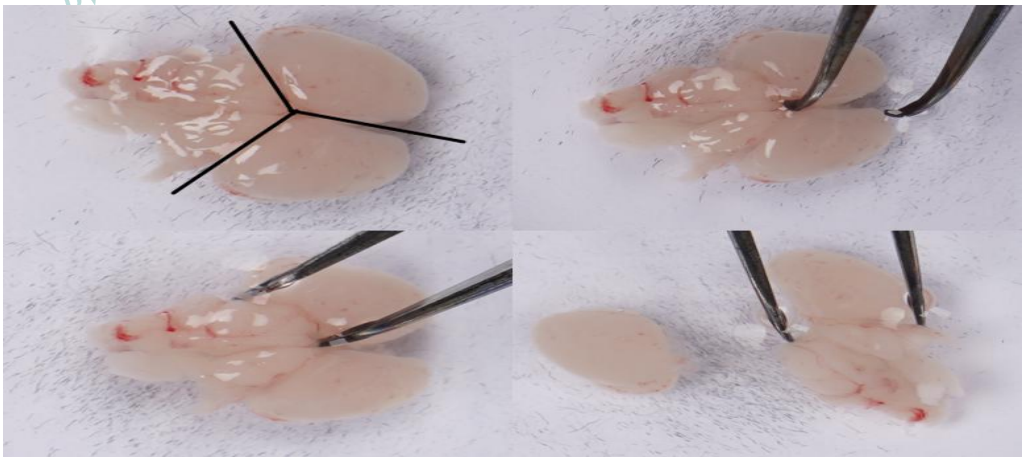


Figure 2 Separate cerebellum and brain

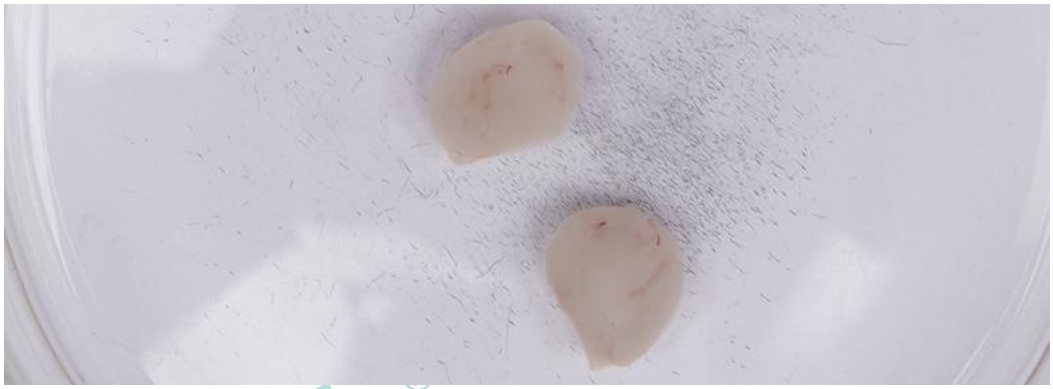


Figure 3 Flip the separated brain



Figure 4 Separate the essence and retain the cerebral cortex



Figure 5 Flip the separated brain again

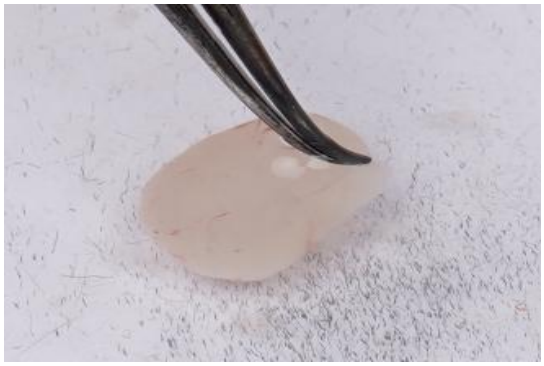


Figure 6 Tear open part of the meninges

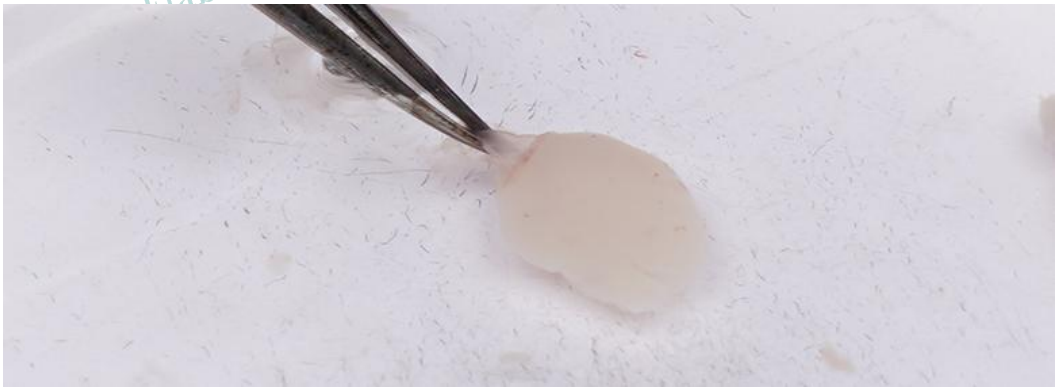


Figure 7 Stabilize the tissue with the left hand, and gently pull to strip off the meninges