Rat Cortical Neuron Isolation and Culture Kit

Cat. No.: P-CA-604

Size: 3Tests / 10Tests

Background

The Rat Cortical Neuron Isolation and Culture Kit is specifically developed to extract primary Rat Cortical Neurons. 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. Through immunofluorescence analysis, the cell purity (β-Tubulin Ⅲ-positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for isolating cortical neurons 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 Cortical Neurons	3Tests (250 mL) 10Tests (500 mL×2)	Faint Yellow Clear Liquid	2-8°C, 1 year
Specialized Digestive Solution For Rat Cortical Neurons	3Tests (24 mL) 10Tests (80 mL)	Colorless Clear Liquid	-5~-20°C, 1 year
Basic Culture Medium For Rat Cortical Neurons	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement For Rat Cortical Neurons	3Tests (2.5 mL) 10Tests (5 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
Digestion Termination Solution For Rat Cortical Neurons	3Tests (75 mL) 10Tests (250 mL)	Red Clear Liquid	2-8°C, 1 year
Planting Solution For Rat Cortical Neurons	3Tests (3 mL) 10Tests (10 mL)	Colorless Clear Liquid	-5~-20°C, 1 year
Screening Solution For Rat Cortical Neurons	3Tests (60 μL) 10Tests (200 μL)	Colorless 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~-20°C (such as Specialized Digestive Solution for Rat Cortical Neurons) can be preserved at 4°C for 30 days after thawing. For long-term storage, aliquot them into single-use portions and freeze at -20°C. Thaw again 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 rats to familiarize operators with procedural workflows and improve tissue dissociation efficiency.
3. During the entire tissue dissociation process, place the small dish containing the tissue on an ice tray/ice box (2-8°C) to maintain hypothermic conditions. Critical precautions: Monitor temperature rigorously to prevent ice crystal formation in tissues/liquids.
4. 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.
5. Cortical Neurons are terminally differentiated cells that lack proliferative capacity and exhibit high fragility. Improper enzymatic digestion can readily induce cell injury. During cell seeding, it is recommended to calculate the seeding density based on experimental requirements and inoculate into corresponding culture vessels. The recommended conversion ratios are as follows: one T25 flask ≈ one 6 cm dish ≈ two wells of a 6-well plate ≈ five to six wells of a 12-well plate.

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).
2) Reagent Thawing and Rewarming:
   ① Specialized Digestive Solution For Rat Cortical Neurons; Supplement For Rat Cortical Neurons; Planting Solution For Rat Cortical Neurons; Screening Solution For Rat Cortical Neurons: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Cortical Neurons; Basic Culture Medium For Rat Cortical Neurons; Digestion Termination Solution For Rat Cortical Neurons: Equilibrate to room temperature.
3) Preparation of complete culture medium: Add 2.5 mL of Supplement For Rat Cortical Neurons into 50 mL of Basic Culture Medium For Rat Cortical Neurons, mix thoroughly.
   Note: Complete culture medium: 2–8°C, 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°C to avoid repeated freeze-thaw cycles.
4) Coating of Culture Vessels: Add 1 mL of Rat Cortical Neurons Planting Solution into a T25 culture flask. Gently swirl to ensure even coverage of the bottom surface. Incubate the flask in a 37°C, 5% CO_2 incubator for 0.5-2 hours.

2. Dissection Protocol:

1) Animal Disinfection and Processing: Spray the animal with 75% medical-grade ethanol for disinfection, and transfer it to a clean bench. Euthanizing the animal via decapitation, and collect the head 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 Cortical Neurons 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 Cortical Neurons (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 Cortical Neurons.

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 Cortical Neurons 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 Cortical Neurons to the centrifuge tube. Mix gently, seal the tube opening with parafilm, and incubate at 37°C, 5% CO_2 for 5 minutes.

② Transfer the tube to a 37°C 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 Cortical Neurons. 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 Cortical Neurons.

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 Cortical Neurons 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 pre-coated T25 cell culture flask and aspirate the Rat Cortical Neurons Planting Working Solution. Slowly add Specialized Washing Solution For Rat Cortical Neurons to wash the flask twice, 5 mL each time, along the inner wall of the flask to avoid disrupting the coated surface. Aspirate the washing solution after gentle rinsing. Resuspend the cell pellet in 5 mL of Complete Medium For Rat Cortical Neurons and transfer the cell suspension into the coated T25 flask. Add 20 µL of Rat Cortical Neurons Screening Working Solution. Incubate at 37°C with 5% CO_2 for static culture.
2) Medium replacement: Perform the first medium replacement at 48 h, followed by subsequent replacements every 2-3 days. Cells typically reach 80-90% confluency within 2–3 days post-seeding, with the purity validated by our laboratory to exceed 90%.

Note: Neuronal cells are highly vulnerable. Prior to medium replacement, preheat the complete culture medium to 37°C in advance to prevent temperature-induced shrinkage and detachment of rat Cortical Neurons.

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°C 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.
Low cell purity	Improper use of screening solution	Verify storage method and dosage of selection medium comply with manufacturer's instructions.
	Incompletely peeling off the meninges	Ensure the age in days of rat pups is maintained at 1-2 days, as older age increases the difficulty of meningeal dissection.
		Ensure thorough removal of meningeal tissues to minimize contamination risks.

Anatomy Images for Reference



Figure 1 Separated brain

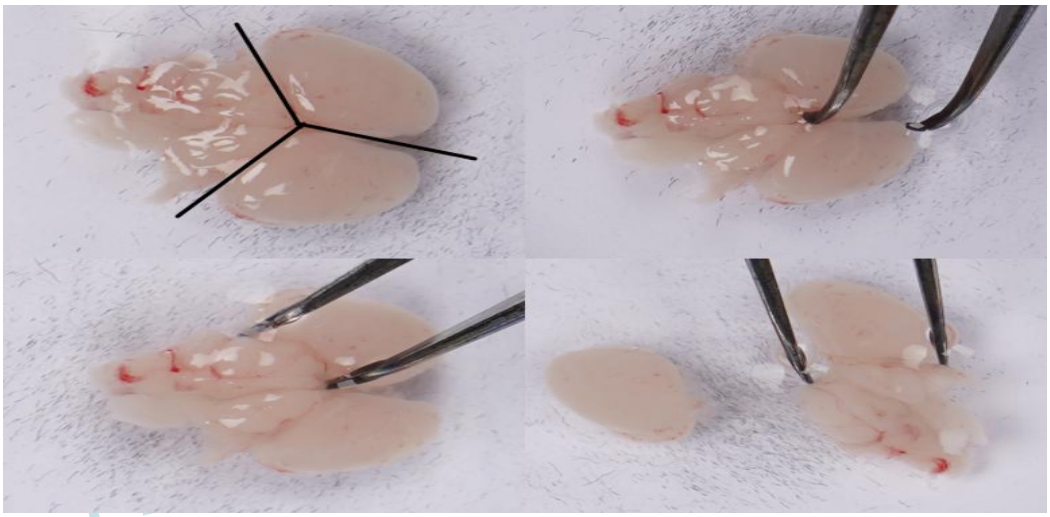


Figure 2 Separate cerebellum and brain



Figure 3 Flip the separated brain



Figure 4 Separate the essence and retain the cerebral cortex



Figure 5 Flip the separated brain again

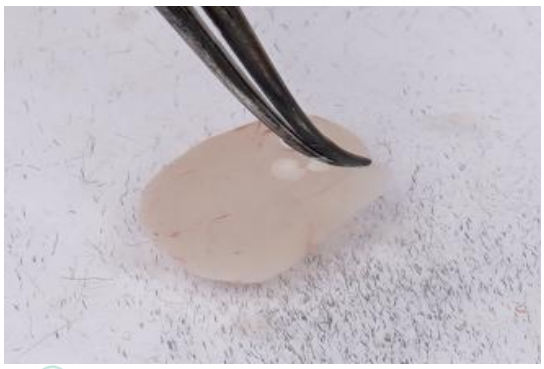


Figure 6 Tear open part of the meninges



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

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