Mouse Brain Microvessel Isolation Kit

Cat. #: P750-4 (4 reactions);

Storage: 4°C **Shelf Life:** 3 months

Product Description

This kit is for mouse brain microvessel isolation. Easy protocol and high yield. It is for research use only.

Product Components

Components	Amount	Storage
Solution A	100 mL (24.75 mL / rxn)	4°C
Solution B	20 mL (5 mL / rxn)	4°C
Pestle	4	Room temperature
Tissue Strainer 1	4	Room temperature
Tissue Strainer 2	4	Room temperature

Important note:

All procedures should be done in a clean station.

Always keep Solution A, B on ice. Always use ice cold Solution A, B for whole experiment.

Protocol:

1. Sit Solution A, B on ice.
2. Euthanize one mouse and take its brain. This protocol is good for half or one whole mouse brain. Rinse the brain with 1x PBS to clean any debris. Dissect **cerebral cortex, hippocampus and stratum of mouse brain**, and put it in 8 mL ice cold Solution A in a 60mm Falcon Petri Dish (Corning #353004).
3. Put the dish under a dissection microscope and remove the meninges as soon as possible.
4. Make homogenate: Transfer the tissue only into a 1.5 mL microcentrifuge tube. Use a pestle (provided) to homogenize the tissue in the microcentrifuge tube on ice or in cold room:
   4.1 First, pestle the tissue **without** adding Solution A for 100 pestlings.
   4.2 Add 0.2 mL Solution A then do another 200 pestlings.
   4.3 Add another 0.2 mL Solution A and do another 100 pestlings.

   A pestle motor can be used to grind the tissue. However, data shows that hand pestling can produce more consistent result than motor pestling.
5. Add 5 mL ice cold Solution B to a 50 mL centrifuge tube and put it on ice.

6. Pour the homogenate from the 1.5 mL microcentrifuge tube (step 4) into the 50 mL centrifuge tube, which containing 5 mL Solution B. Add 0.4 mL Solution A to the 1.5 mL microcentrifuge tube to rinse, then pour it into the 50 mL centrifuge tube. Repeat the rinse-pour for another 3 times. The total volume in the 50 mL centrifuge tube is 7 mL now (0.2 mL + 0.2 mL + 5 mL + 0.4 mL x 4).
7. Add another 3 mL Solution A into the same 50 mL centrifuge tube. The total volume is 10 mL now. Mix well by vortex, then centrifuge at **4,000 rpm for 10 minutes at 4°C.**
8. Now you can see a fatty layer on the top in the 50 mL centrifuge tube. Remove the fatty layer carefully using 1 mL pipet tips (Cut the end of tip to make a wider opening. This makes the fat removing easier). Now, after removing the fatty layer, there should be 7–7.5 mL homogenate left in the 50 mL centrifuge tube.
9. Add an equal volume of Solution A (7–7.5 mL) into the 50 mL centrifuge tube, and vortex briefly to mix well. Then centrifuge at **4,000 rpm for 10 minutes at 4°C**.
10. Aspirate and discard the supernatant. Add 2mL Solution A to the pellet and Re-suspend it well by pipetting up and down.
11. Put a **Tissue Strainer 1** on a new 50 mL centrifuge tube, then pass the suspension from step 10 through the Tissue Strainer 1 to collect the filtrate.
12. Pass the collected filtrate through a **Tissue Strainer 2** on another container. Now the mouse brain microvessels are retained on the **Tissue Strainer 2**.
13. Wash the **Tissue Strainer 2** with 0.75 mL Solution A once. Be careful, wash evenly and gently to minimize the loss of some microvessels.
14. To collect the microvessels, flip over the **Tissue Strainer 2** and put it on a 35mm culture dish (Corning #430165). Use 1.5 mL Solution A to wash down the microvessels from the **Tissue Strainer 2** to the 35x10mm culture dish.

    Now you have the isolated mouse brain microvessels suspended in 1.5 mL Solution A. Transfer all suspension to a **new** 1.5 mL microcentrifuge tube from the 35mm culture dish. Now the mouse brain microvessel isolation experiment is finished. Centrifuge the 1.5 mL tube at **5,000rpm for 10 minutes at 4°C** to collect pellet for downstream protein or/and RNA extraction or do it later by storing at -80°C for up to one month.

    For downstream experiment:

    Use our "Tissue RNA Storage Solution, Cat.# W0592" to store microvessels for up to 1 year.

    Use our "3-min. Total Protein Extraction Kit, Cat.# P502" to extract protein.

    Use our "3-min. Detergent-free Total Protein Extraction Kit, Cat.# P506" to extract detergent-free protein.

    Use our "40-min. Plasma Membrane Protein Extraction Kit, Cat.# P503" to extract membrane protein.