Minute™ Synaptosome Isolation Kit

Catalog Number: SY-052

Description

The ability to isolate synaptosomes from neuronal tissues/cultured cells is essential for understanding the mechanisms of neurological diseases. Contained in synaptosomes are synaptic vesicles with diameters of 80-200 nm that play an important role in signal transmission. Traditionally, synaptosomes are isolated by density ultracentrifugation. The procedure is relatively tedious and time-consuming. A larger amount of starting material is usually required. Minute™ synaptosome isolation kit provides a simple and rapid method for isolating synaptosomes from fresh/frozen neuronal tissues/cultured cells. The protocol can be completed in less than 1 h without using Dounce homogenizer and ultracentrifugation. The amount of starting material required (10-50 mg tissue) is only a fraction of that required by the traditional method. The buffers used are detergent-free and synaptosome-associated proteins are isolated in native form.

Kit Components (50 prep)

1.	Buffer A	25 ml
2.	Buffer B	3 ml
3.	Buffer C	25 ml
4.	Plastic rods	2
5.	Filter Cartridges	50
6.	2ml collection tubes	50

Additional Materials Required

1 X PBS, Vortexer, and Tabletop centrifuge with maximum speed of 16,000 X g.
(**The centrifuge should be able to reach maximum speed within 10 seconds)**

Important Information:

1. **All centrifugation steps should be performed at 4-8°C in a cold room or in a refrigerated centrifuge.**
2. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to aliquot of buffer A and buffer C prior to use. If protein degradation is a concern, add protease inhibitor cocktails to aliquot of buffer A prior to use.
3. It is recommended to use BCA Protein Assay Kit (Pierce) for determination of protein concentration.

Protocol

1. Place desired number the filter cartridges in collection tubes and put on ice. Pre-chill buffer A and C on ice prior to use.
2. **For Brain Tissue Sample**, place ~30 mg (ranging from 10-50 mg/sample) fresh or frozen tissue in a filter cartridge. Add 200 µl buffer A to the cartridge and grind the tissue with the plastic rod by pushing the tissue against the surface of the filter repeatedly with twisting force for 1-2 min.

Add 300 µl buffer A to the same filter cartridge. Go to step 3.
(The plastic rod is reusable. Clean with 70% alcohol or water).

For cultured cells, collect 30-40 X 10^6 cells by low-speed centrifugation (500-600 X g for 5 min). Wash cells once with 1 ml cold PBS. Remove supernatant completely and resuspend the pellet in 500 µl buffer A. Incubate the cell suspension on ice for 5 min. Go to step 3.

3. Cap the filter cartridge, invert a few times and centrifuge at 16,000 X g for 15-20 seconds (for cultured cells, the pass through can be repassed through the same filter to increase the final yield if desired).
4. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds.
   Centrifuge at 1500 X g for 5 min. The pellet contains nuclei, large cell debris and some un-ruptured cells. Carefully transfer supernatant to a fresh tube and mix with 50 ul buffer B by vortexing for 20-30 seconds. Centrifuge at 13000 X g for 15 min. Remove supernatant (cytosolic fraction).
5. Resuspend the pellet in 0.5 ml buffer C by pipetting up and down repeatedly. Incubate on ice for 5 min. After incubation, vortex vigorously for 10-20 seconds and centrifuge at 2000 X g for 5 min (the pellet contains mainly plasma membrane and other lipids). Transfer supernatant to a fresh tube.
6. Centrifuge at 13000 X g for 20 min. The pellet contains isolated synaptosomes. The pellet can be resuspended in 100-300 ul buffer that is compatible with downstream experiment (the amount is depending upon the amount of starting material and size of the pellet). The pellet can also be resuspended in reagent list in the following table.

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.