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ProductInformation

Synaptic Vesicles Isolation Kit

Catalog Number **SV0100**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Synaptic vesicles are spherical particles with diameters of 50-200 nm, which contain a variety of neurotransmitters and are involved in neuronal communication. Synaptic vesicles are concentrated at the nerve terminals. Synaptic transmission involves the release of neurotransmitters from presynaptic nerve terminals, via Ca^{2+} triggered exocytotic fusion of synaptic vesicles with the presynaptic plasma membrane, and their subsequent binding to specific postsynaptic receptors.[1] After exocytosis, synaptic vesicle membranes and protein constituents are retrieved from the plasma membrane by endocytosis and locally recycled for storage and future use.[2] Vesicular proteins can be divided into two groups according to their function, the trafficking proteins and the proteins involved in neurotransmitter uptake and storage.[3-5]

The Synaptic Vesicles Isolation Kit provides all the reagents and a comprehensive procedure required for the preparation of an enriched synaptic vesicle fraction. The isolation procedure involves tissue homogenization, hypo-osmotic lysis of the synaptosomes, and differential centrifugation[6] followed by an OptiPrep® density gradient.[7] The kit also contains an antibody specific for synaptophysin, a synaptic vesicle marker, which can be used to follow the enrichment procedure.

The kit was tested with rat and rabbit brain tissues.

Components

The kit is sufficient for the preparation of synaptic vesicles from 15 rat brains (1.5–2 g per brain).

Homogenization Buffer 5× — 125 ml
Catalog Number H1789

Lysis Buffer 5× — 60 ml
Catalog Number L8417

Storage Buffer for Synaptic Vesicles 5× — 30 ml
Catalog Number S7445

OptiPrep Density Gradient Medium — 100 ml
Catalog Number D1556
60% (w/v) solution of iodixanol in water

Monoclonal Anti-Synaptophysin — 100 µl
Clone SVP-38, Ascites fluid
Catalog Number S5768

Equipment and Reagents Required But Not Provided

- Sorvall® RC-5C centrifuge with SA-600 head or equivalent
- Centrifuge tubes
- Ultracentrifuge with 45Ti (Beckman Coulter®, fixed angle) and TFT 65.13 (Kontron® Instruments, fixed angle) heads or equivalent, and tubes suitable for volumes of 40 ml and 13.5 ml
- For homogenization of brain tissue - Overhead electric stirrer (e.g., Heidolph RZR 2050) together with Potter-Elvehjem PTFE pestle and glass tube homogenizer - 45 ml (Catalog Number P7984) or equivalent
- For homogenization of crude synaptic vesicle extract - Potter-Elvehjem PTFE pestle and glass tube homogenizer - 8 ml (Catalog Number P7859) or equivalent
- Ultrapure water (17 MΩ·cm or equivalent)

Precautions and Disclaimer

The Synaptic Vesicles Isolation Kit is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Use ultrapure water (17 MΩ·cm or equivalent) for reagent preparation.

1× Homogenization Buffer - Dilute an aliquot of the Homogenization Buffer 5× (Catalog Number H1789) 5-fold with water. Keep the 1× Homogenization Buffer at 2–8 °C until use.
Note: Suggested volume of 1× Homogenization Buffer for processing 1 rat brain (1.5–2 g of tissue) is 40 ml.

1× Lysis Buffer - Dilute an aliquot of the Lysis Buffer 5× (Catalog Number L8417) 5-fold with water. Keep the 1× Lysis Buffer at 2–8 °C until use.
Note: Suggested volume of 1× Lysis Buffer for processing 1 rat brain (1.5–2 g of tissue) is 20 ml.

1× Storage Buffer - Dilute an aliquot of the Storage Buffer for Synaptic Vesicles 5× (Catalog Number S7445) 5-fold with water. Keep the 1× Storage Buffer at 2–8 °C until use.
Note: Suggested volume of 1× Storage Buffer for 1 processed rat brain (1.5–2 g of tissue) is 4 ml.

40% OptiPrep Solution - Mix 0.4 ml of Storage Buffer for Synaptic Vesicles 5× (Catalog Number S7445) with 0.8 ml of water and 0.8 ml of OptiPrep Density Gradient Medium (Catalog Number D1556).
Note: Suggested volume of 40% OptiPrep Solution is 2 ml for 1 rat brain (1.5–2 g of tissue).

Storage/Stability
Store the unopened kit at –20 °C.
Upon first use, store the anti-synaptophysin antibody (Catalog Number S5768) in working aliquots at –20 °C.
The rest of the kit components can be stored at 2–8 °C.

Procedure
Synaptic vesicles can be easily prepared from rat brain tissue by a simple method of homogenization followed by synaptosome hypo-osmotic lysis and differential centrifugations.[6] The pellet obtained represents a crude synaptic vesicle fraction. A further enriched synaptic vesicle fraction is obtained by an OptiPrep density gradient.

The differential centrifugation steps include:
1. Low speed centrifugation (1,500 × *g*) to pellet brain tissue debris after tissue homogenization.
2. Medium speed centrifugation (20,000 × *g*) to pellet intact and lysed synaptosomes.
3. High speed centrifugation (70,000 × *g*) to pellet the crude synaptic vesicle fraction.
4. High speed centrifugation (~207,000 × *g*) for the OptiPrep density gradient.

A flow diagram for the preparation of synaptic vesicles is shown in Appendix II.

I. Preparation of Crude Synaptic Vesicles from Rat Brain Tissue
Brain tissues from different animals may give different yields of synaptic vesicles, as they vary in parameters such as size, fat content, etc. When using rabbit brain tissue, it is recommended to cut the brain into several pieces in order to achieve a better homogenization.

1. Weigh the brain (1.5–2 g of brain tissue is obtained from a rat weighing 150–200 g).
2. Homogenize the tissue in 1× Homogenization Buffer (10 ml of buffer per 1 g of brain tissue) with PTFE pestle in 40 ml glass tube homogenizer.
3. Centrifuge the homogenate at 1,500 × *g* for 10 minutes at 2–8 °C (3,220 rpm using Sorval SA-600 rotor).
4. Collect the supernatant and keep on ice.
5. Resuspend the pellet in the same volume of 1× Homogenization Buffer as in step 2.
6. Centrifuge the resuspended pellet at 1,500 × *g* for 10 minutes at 2–8 °C (3,220 rpm using Sorval SA-600 rotor).
7. Collect the supernatant and combine it with the supernatant from step 4.
8. Centrifuge the combined supernatants at 20,000 × *g* for 20 minutes at 2–8 °C (11,760 rpm using Sorval SA-600 rotor).
9. Discard the supernatant.
10. Homogenize the pellet in 1× Lysis Buffer (10 ml of buffer per 1 g of brain tissue). Use PTFE pestle in 40 ml glass tube homogenizer.
11. Keep the homogenate on ice for 45 minutes.
12. Centrifuge the homogenate at 20,000 × *g* for 20 minutes at 2–8 °C (11,760 rpm using Sorvall SA-600 rotor).
13. Collect the supernatant and centrifuge it in an ultracentrifuge at ~70,000 × *g* for 45 minutes at 2–8 °C (30,000 rpm using Type 45Ti rotor, Beckman Coulter, fixed angle).
14. Discard the supernatant.
15. Homogenize the pellet in 1× Storage Buffer (2 ml of buffer per 1 g of brain tissue). Use PTFE pestle in 8 ml glass tube homogenizer.
16. Use this crude synaptic vesicle extract immediately or store it at 2–8 °C for further use.

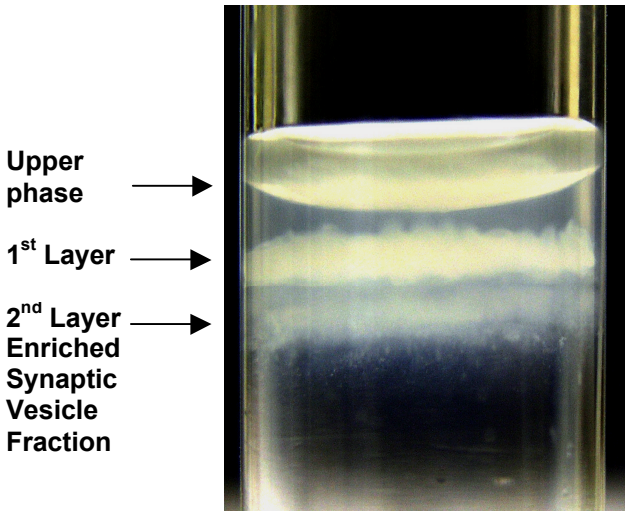
Note: Synaptic vesicle preparations can be stored at 2–8 °C for a few days. The synaptic vesicles will lose some uptake activity with time (~20% after one week at 2–8 °C). It is not recommended to store the synaptic vesicles at –70 °C if uptake assays are to be performed.

II. Enrichment of Synaptic Vesicles on a Density Gradient

This density gradient centrifugation is for obtaining an enriched synaptic vesicle fraction from the crude extract. The procedure uses a 13.5 ml ultracentrifuge tube.

1. Dilute 2 ml of crude synaptic vesicle extract with 2 ml of OptiPrep Density Gradient Medium (Catalog Number D1556) and vortex vigorously.
2. Place the mixture from step 1 in a 13.5 ml ultracentrifuge tube.
3. Overlay the mixture with 0.8 ml of the prepared 40% OptiPrep Solution.
4. Centrifuge at ~207,000 × *g* for 3 hours at 2–8 °C (55,000 rpm using Centrikon fixed angle TFT 65.13 rotor, Kontron). Brake to 800 rpm and then let stop without brake. After centrifugation, two phases will appear in the tube. The second layer of the lower phase consists of the enriched synaptic vesicle fraction (Figure 1).

Figure 1.
Post OptiPrep Density Gradient Layering



The second layer of the lower phase consists of the enriched synaptic vesicle fraction.

5. Carefully aspirate off the upper phase and the first layer of the lower phase.
6. Carefully withdraw the second layer of the lower phase containing the **enriched synaptic vesicle fraction**. Use this fraction immediately or store it at 2–8 °C for further use.
   Note: Synaptic vesicle preparations can be stored at 2–8 °C for a few days. It is not recommended to store the synaptic vesicles at –70 °C if uptake assays are to be performed.
7. Determine protein concentration using Bradford method.

III. Immunoblot Analysis of the Crude and Enriched Synaptic Vesicle Fractions

In order to check for the presence of synaptic vesicles in the crude and enriched fractions, and confirm the enrichment step, perform an immunoblotting assay using the anti-synaptophysin antibody, which is specific for the vesicular membrane bound protein synaptophysin.[8]

Immunoblotting procedure

1. Dilute an aliquot of each fraction to a protein concentration of 50–100 µg/ml.
2. Load the diluted samples on an SDS-PAGE gel (10–12%), run the gel, and then transfer the proteins to a nitrocellulose membrane.
3. Block the nitrocellulose membrane using an appropriate blocking solution.
4. After blocking the membrane, incubate it for 2 hours at room temperature (or alternatively, overnight at 2–8 °C) with a 1:1,000 dilution of the Monoclonal Anti-Synaptophysin antibody (Catalog Number S5768) in blocking buffer. For best results with different preparations it is recommended to determine the optimal working dilutions by antibody dilution titration test. During this incubation step the membrane should be gently agitated.
5. Continue according to the protocol of the ProteoQwest™ Chemiluminescent Western Blotting Kit (Catalog Number PQ0201) or an equivalent kit.
6. Expose the membrane to an X-ray film.

References

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Appendix I
Procedure for Radiolabeled 5-HT (Serotonin) Uptake Assay

In order to check whether the enriched vesicles are still intact, an assay was developed for the measurement of radiolabeled 5-HT (serotonin) uptake. Intact synaptic vesicles incubated with radiolabeled 5-HT, in the presence of ATP, can accumulate the radioactive neurotransmitter according to the electrochemical gradient, produced by the H^+-ATPase pump. After filtration through a glass microfiber filter, the sample is washed to remove residual radioactive material, while the neurotransmitter containing vesicles remain on the filter. The radioactive 5-HT accumulated in the synaptic vesicles is then determined by counting the filter in a scintillation counter. The net uptake of the assayed extract is obtained by the comparison between two samples: one containing ATP and the other not containing ATP (background).

Reagents Needed But Not Provided

- Radiolabeled 5-HT (^{3}H-5HT, 5-Hydroxy[G-^{3}H]-tryptamine creatinine sulphate)
- 1 M $MgCl_2$ (Catalog Number M1028)
- ATP Magnesium salt (Catalog Number A9187)
- KOH (Catalog Number P1767)
- Ultima Gold™ Liquid scintillation cocktail (Catalog Number L8286)

Equipment Needed

- 12 × 75 mm disposable culture tubes or equivalent
- Water bath (30 °C)
- Whatman® glass microfiber filters, Grade GF/C, diameter 2.5 cm (Catalog Number Z242349)
- Filter funnel
- Vacuum pump
- Scintillation counter
- Scintillation vials and caps (Catalog Number Z376825)

Reagent preparation
Prepare all reagents with ultrapure water (17 MΩ·cm or equivalent)

1.25 mM $MgCl_2$ Solution - Dilute 1 M $MgCl_2$ (Catalog Number M1028) 800-fold to a concentration of 1.25 mM in water.

1× Assay Mixture (10 mM Potassium Phosphate, pH 7.4, containing 110 mM KCl with 1 mM $MgCl_2$) - Dilute the Storage Buffer for Synaptic Vesicles 5× five-fold with the 1.25 mM $MgCl_2$ Solution.

40 mM K-ATP Solution (pH 7.4) - Dissolved the ATP in water to 40 mM final concentration and titrate with 0.5 N KOH to pH 7.4.

Reaction Mixture - Dilute the ^{3}H-5HT [1 mCi/ml] 30-fold in 1× Assay Mixture (210 µl of 1× Assay Mixture and 7 µl of 1 mCi/ml ^{3}H-5HT, should be enough for 8 samples).

Procedure
Work in duplicates.

1. Into two tubes (~6 ml), add 10 µl of 40 mM K-ATP Solution. Mark these tubes "test".
2. Into two tubes (~6 ml), add 10 µl of 1× Assay Mixture. Mark these tubes "background".
3. Add to each tube a volume of the **enriched synaptic vesicle fraction** equivalent to at least 20 µg of protein in a total volume of 200 µl. If required, bring the total volume to 200 µl with 1× Assay Mixture.
4. Vortex gently and pre-incubate the samples at 30 °C for 10 minutes.

From this point forward, work with one tube at a time. This makes it is easier to perform the procedure and does not affect the results.

5. Add 25 µl of the Reaction Mixture to the tube.
6. Vortex and incubate the sample at 30 °C for 2 minutes.
7. Stop the reaction by adding 5 ml of ice-cold 1× Assay Mixture.
8. Filter through GF/C filters using vacuum.
9. Wash the tube with 5 ml of ice-cold 1× Assay Mixture and filter it through the GF/C filter once again.
10. Dry the GF/C filters and while drying, proceed to process the next tube (from steps 5-10).
11. Transfer the dried filters into scintillation vials and add 4 ml of scintillation cocktail to each vial. **Cap the vials!**
12. Read cpm in a scintillation counter.

Results
Subtract the average cpm of the "background" samples from the average cpm of the "test" samples. This indicates the net uptake of the samples.

Appendix II
Synaptic Vesicle Preparation Flow Chart