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ProductInformation

ANTI -SYNAPTOTAGMIN
Developed in Rabbit
IgG fraction of Antiserum

Product No. **S 2177**

Product Description

Anti-Synaptotagmin (p65) is developed in rabbit using a synthetic peptide MVSASHPEALAAPVTT-K corresponding to the N-terminus of synaptotagmin I (SytI) of rat origin (amino acids 1-16 with C-terminally added lysine) conjugated to KLH as immunogen. This sequence is highly conserved among species (mouse, chicken, bovine and human SytI) and is not found in other known synaptotagmin isoforms (SytII-VIII). Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum which is essentially free of other rabbit serum proteins.

Anti-Synaptotagmin reacts specifically with synaptotagmin (Syt, p65), derived from rat brain tissue (65 kDa). The antibody may be used in immunoblotting of rat brain extract. Staining of synaptotagmin band is specifically inhibited with synaptotagmin peptide (rat, amino acids 1-16 with C-terminally added lysine).

Synaptotagmin (Syt, p65) is an abundant synaptic vesicle (SV) membrane protein.[1, 2] It is characterized by a short intravesicular N-terminal domain, a single transmembrane region and two copies of highly conserved internal repeats, known as the C2A and C2B domains, which are homologous to the C2 regulatory region of protein kinase C (PKC) in the cytoplasmic domain. [2,3] Synaptotagmin binds Ca^{2+} phospholipids with high affinity and has a central role in Ca^{2+} regulated neurotransmitter release. Synaptotagmin functions as a Ca^{2+} sensor and is required for efficient exocytosis, particularly in the vesicle docking and/or fusion step with the plasma membrane. [4,5] The molecular events leading to SV docking, activation and fusion/exocytosis are complex steps involving multiple interacting proteins. [7-8] It has been suggested that during synaptic exocytosis an initial core complex (also called the SNAP receptor or SNARE complex) is formed between synaptobrevin/VAMP (v-SNARE) on the synaptic vesicle and SNAP-25 and syntaxin on the target plasma membrane (t-SNAREs). The core complex serves as a receptor for two additional, membrane fusion proteins: bSNAP and NSF which interact with the “core complex” leading to initiation of fusion and core complex dissociation. Ca^{2+} influx triggers synaptotagmin to interact with either syntaxin or SNAP-25 and the cytoplasmic domain of neurexin leading to fusion and exocytosis. The C2A domain of Syt has been implicated in the vesicular fusion step, whereas its C2B domain appears to be involved in synaptic vesicle recycling. Several lines of evidence suggest that SytI is essential as a Ca^{2+} sensor in triggering neurotransmitter release. Mutations or deletion of synaptotagmin result in severely impaired Ca^{2+} triggered neurotransmitter release. Synapses of SytI knockout mice lack the fast-component of Ca^{2+} dependent neurotransmitter release, but exhibit no changes in the slow, Ca^{2+} independent component of synaptic vesicle exocytosis.[9] At least eight different isoforms of synaptotagmin (SytI-VIII) are expressed in the brain, four of which (SytIV, V, VII and VIII) are also expressed in non-neuronal tissues.[10,11] The synaptotagmins isoforms have different Ca^{2+} binding affinities, are differentially expressed in neurons, with partly overlapping patterns, suggesting that different synaptotagmins may have distinct functions. Antibodies that react specifically to synaptotagmin are useful for elucidating the expression of these isoforms in a variety cell types and tissues.

Reagents

The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as preservative.

Precautions

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Product Profile

A minimum working dilution of 1:5,000 is determined by immunoblotting using extract of rat brain membrane fraction.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Storage

For continuous use, store at 2-8 °C for up to one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

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Immunoblotting Procedure of Rat Brain Membrane Fraction Extract

Reagents and Equipment

1. Rats (Sprague-Dawley) (brain trissue).
2. Phenylmethylsulfonyl fluoride (PMSF) (Product No. P 7626), 0.5M in ethanol.
3. Pepstatin A (Product No. P 4265), 2mg/ml in DMSO.
4. Leupeptin (Product No. L 2884).
5. Aprotinin (Product No. A 4529).
6. Dithiothreitol (DTT) (Product No. D 9760).
7. Homogenization Buffer (Rat brain), Ice Cold: 20 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose, 10 mM EGTA, 2 mM EDTA, and protease inhibitors: 2 mM PMSF, 50 mg/ml leupeptin, 25 mg/ml aprotinin, 10 mg/ml pepstatin A and 2 mM DTT.

Note: Add all protease inhibitors freshly before tissue homogenization.

8. Homogenizer: Mechanical homogenizer.
9. Refrigerated High-Speed Centrifuge.
10. Protein assay kit .
11. Laemmli sample (1X) buffer containing 2-mercapto-ethanol.

Preparation of Rat Brain Membrane Fraction Extract

Note: All procedure steps are carried out on ice, unless noted otherwise.

1. Rapidly dissect out whole brains (5 g) from Sprague-Dawley rats (4-5 rats, 250-300g) and collect into ice cold homogenization buffer.
2. Homogenize tissue in 5x volumes of ice cold homogenization buffer (w/v) using mechanical homogenizer at maximum speed (3 x 10 sec pulses with 1 min rest in between).
3. Homogenize briefly on ice with fine glass homogenizer.
4. Centrifuge homogenate at 1,000 x g for 10 min at 4 °C. Collect supernatant.
5. Centrifuge supernatant at 10,000 x g for 20 min at 4 °C. Collect clear supernatant and retain membrane pellet (P2 fraction).
6. Remove 0.5 ml aliquot of supernatant for protein determination using the Bradford method with BSA as standard.
7. Add to membrane pellet 1x Laemmli sample buffer Boil sample for 5 minutes.
8. Centrifuge sample at 10,000 x g for 5 min. at room temperature. Collect supernatant and discard insoluble pellet.
9. Aliquot samples of rat brain membrane extract and store at –70 °C.

Reagents and Equipment for Immunoblotting

1. Rat brain membrane fraction extract.
2. 10% polyacrylamide slab minigel with 5% stacking gel restained HMW markers (Product No.C 3312).
3. Blocking Buffer: 10% dry milk (w/v) in 10 mM phosphate buffered saline (PBS), pH 7.4.
4. Dilution Buffer: 1% BSA in PBS pH 7.4 containing 0.05% Tween-20.
5. Washing Buffer: PBS pH 7.4 containing 0.05% Tween-20.
6. Synaptotagmin I (SytI) peptide (amino acids 1-16 with C-terminally added lysine).
7. Dissolve in distilled deionized water at 0.5 mg/ml. Store aliquots at –20 °C.
8. Primary antibody: IgG fraction Anti-Synaptotagmin (rb) at appropriate dilution in dilution buffer.
9. Secondary Antibody: Alkaline Phosphatase Anti-rabbit IgG (gt) (Product No. A 9919) at diluted in dilution buffer.
10. Substrate: BCIP/NBT Tablets (Product No. B 5655).
11. Electrophoresis and transfer apparatus.

Immunoblotting Procedure

Note: In order to obtain best results in different preparations it is recommended to optimize procedure conditions (antibody dilutions, incubation times,blocking conditions etc.), for a specific application.

1. Resolve rat brain membrane extract (200 ml/slab) on precast 10% polyacrylamide minigel.
2. Run SDS-PAGE at room temperature.
3. Perform transfer for 1 hour at room temperature to nitrocellulose membrane.
4. Block nitrocellulose membrane in blocking buffer for at least 1 hour at room temperature.
5. Incubate membrane with primary antibody dilutions for 2 hours at room temperature [(a)].
6. Wash membrane with washing buffer 4 x 5 min.
7. Incubate membrane with secondary antibody at recommended dilution in dilution buffer for 1 hour at room temperature.
8. Wash membrane with washing buffer 4 x 5 min. Wash 1 x 5 min. in deionized water.
9. Dissolve BCIP/NBT substrate tablet each in 10 ml deionized water. Incubate membrane with substrate solution.
10. Wash membrane thoroughly with deionized water.
11. Air-dry blots on filter paper.

(a)Note: For specific inhibition of synaptotagmin (Syt) band (65 kDa) it is recommended to incubate prediluted antibody with synaptotagmin peptide (1-16) , 10 mg/ml, (final concentration), for 2 hours at room temperature or overnight at 4 °C.

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