RecombinantGFP/SNAP25B/VAMP-2

Catalog Number: 7375-SV

DESCRIPTION

Source

E. coli-derived GFP/SNAP25B/VAMP-2 protein

MVKSADI	10-His tag	Linker 1	GFP (Ser2-Lys238) Accession # ACS44347	Linker 2	Human SNAP25B (Asn93-Gly206) Accession # P60880	Human VAMP-2 (Ser2-Lys94) Accession # P63027	Cys

N-terminus C-terminus

N-terminal Sequence Analysis	Val (of MVKSADI)
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	54-58 kDa, reducing conditions
Activity	Measured by cleavage by botulinum and tetanus toxin light chains. >50% of 1 µg can be cleaved by 4 ng of toxin light chain, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>85%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Tween®. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, pH 6.5
- Recombinant *C. botulinum* BoNT-D Light Chain (rBoNT/D-LC) (Catalog # 6037-ZN)
- Substrate: Recombinant GFP/SNAP25B/VAMP-2 (R&D Systems, Catalog # 7375-SV)
- SDS-PAGE and silver stain reagents or Western blot with appropriate antibodies

Assay

1. Dilute Substrate to 100 µg/mL in Assay Buffer.
2. Dilute rBoNT/D-LC to 0.4 µg/mL in Assay Buffer.
3. Combine equal volumes of diluted Substrate with diluted rBoNT/D-LC. Prepare two controls by combining equal volumes of diluted Substrate with Assay Buffer.
4. Incubate reaction vials at room temperature for 1 hour. Incubate one control at room temperature and the other at -20 °C for 1 hour.
5. After incubation, combine reaction mixtures and controls with reducing SDS-PAGE sample buffer at a 2:1 (reaction mixture:sample buffer) ratio (v/v) to stop reactions.
6. Analyze the cleavage products by SDS-PAGE (Load 30 µL of the mixture from step 5 per lane (1 µg Substrate per lane) followed by silver staining and/or Western blot.

Final Assay Conditions

Per Lane:

- rBoNT/D-LC: 4 ng
- Substrate: 1 µg

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Botulinum and tetanus neurotoxins (BoNTs and TeNT) are zinc metalloproteases that hydrolyze and inactivate proteins necessary for neurotransmission. The protease domain is located in the light chain of the neurotoxin. Among the known substrates of BoNTs and TeNT are synaptosomal protein-25 (SNAP25) and vesicle-associated membrane protein (VAMP). This substrate incorporates a green fluorescent protein (GFPuv) and portions of human SNAP25B and VAMP-2 that contain the cleavage sites of all the known BoNTs and TeNT. The light chains of BoNTs -A, -C, and -E cleave the SNAP25B sequence, while BoNTs -B, -D, -F, -G, and TeNT cleave within the VAMP-2 sequence. The substrate can be used in a SDS-PAGE gel-shift assay to detect cleavage by the neurotoxin proteases. Alternatively, the substrate can be coupled to maleimide-activated microwell plates through the C-terminal cysteine residue to generate a high throughput assay format. A similar substrate and assay format has been used to screen for inhibitors of these neurotoxin proteases (1).

References:

1. Hines, H.B. *et al.* (2008) Applied and Environ. Microbiol. **74**:653.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449