**DESCRIPTION**

**Source**
E. coli-derived Met1-Phe425, with an N-terminal Met and 6-His tag
Accession # Q45894

**N-terminal Sequence Analysis**
Met

**Predicted Molecular Mass**
50 kDa

**SPECIFICATIONS**

**SDS-PAGE**
52 kDa, reducing conditions

**Activity**
Measured by its ability to cleave the fluorogenic peptide substrate, SNAPtide. The specific activity is >1 pmol/min/µg, as measured under the described conditions.

**Endotoxin Level**
<1.1 EU per 1 µg of the protein by the LAL method.

**Purity**
>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**Formulation**
Supplied as a 0.2 µm filtered solution in Tris, NaCl and Tween® 20. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**
- Assay Buffer: 50 mM HEPES, 0.05% (v/v) Tween® 20, pH 7.5
- Recombinant C. botulinum BoNT-A Light Chain (rBoNT/A-LC) (Catalog # 4489-ZN)
- Substrate: SNAPtide (FITC/DABCYL) (List Biological Laboratories, Inc., Catalog # 521), 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Spectra Max Gemini EM by Molecular Devices) or equivalent

**Assay**
1. Dilute rBoNT/A-LC to 10 µg/mL in Assay Buffer.
2. Dilute Substrate to 10 µM in Assay Buffer.
3. Load into a black well plate 50 µL of 10 µg/mL of rBoNT/A-LC and start the reaction by adding 50 µL of 10 µM Substrate. As a Substrate Blank combine 50 µL of 10 µM Substrate and 50 µL of Assay Buffer.
4. Read at excitation and emission wavelengths of 490 nm and 523 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

   \[
   \text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}}^* (\text{RFU/min}) \times \text{Conversion Factor}^* (\text{pmol/RFU})}{\text{amount of enzyme (µg)}}
   \]

   

   *Adjusted for Substrate Blank
   **Derived using calibration standard Unquenched Calibration Peptide (List Biological Laboratories, Catalog # 528).

**Final Assay Conditions Per Well:**
- rBoNT/A-LC: 0.50 µg
- Substrate: 5 µM

**PREPARATION AND STORAGE**

**Shipping**
The product is shipped with polar packs. 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, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

**BACKGROUND**
Botulinum neurotoxin type A is one of the seven serotypes of Botulinum Neurotoxins (BoNTs) produced by various strains of Clostridium botulinum (1, 2). BoNTs are synthesized as inactive single chain protein precursors and activated by proteolytic cleavage to generate disulfide-linked two-chain proteins. The 50 kDa light chain contains the catalytic domain, whereas the 100 kDa heavy chain contains an internal translocation domain and a receptor binding domain (3). BoNTs are the most potent protein toxins for humans. As zinc proteases, they cleave SNARE proteins to elicit flaccid paralysis in botulism by blocking acetylcholine release at the neuromuscular junction (2-4). E. coli-expressed recombinant light chains are active proteases. However, they are not toxic because they cannot enter into host cells in the absence of the heavy chains.

**References:**