

[0026] The invention also relates to a toxin assay kit comprising: (1) an assay component according to the invention; (2) an antibody according to the invention which is identified immunohistochemically; and (3) an assay substrate.

[0027] Other embodiments and advantages of the invention are set forth in part in the description, which follows, and in part, may be obvious from this description, or may be learned from the practice of the invention.

DESCRIPTIONS OF THE DRAWINGS

[0028] FIG. 1. A diagram representing the mode of action of the BoNT/A cleavage sensitive antibodies (BACS). BACS antibodies bind across the BoNT/A cleavage site on SNAP-25. This binding event can be used to generate a measurable signal which correlates with the concentration of the full length protein substrate. BoNT/A cleavage destroys the BACS antibody epitope resulting in loss of immunoreactivity and loss of signal.

[0029] FIG. 2. A diagram showing the three designed peptides used for vaccination span the BoNT/A cleavage site in SNAP-25. Sequences shared between peptides are shown in light-gray highlights, while N-terminal and C-terminal additions are shown as underlined and dark-gray, respectively.

[0030] FIG. 3. Photographs of Western blot analysis of N-terminal antibody (SMI-81) and BACS antibodies. Four of the antibodies (indicated by arrows on the figure) only bind full length SNAP-25 by comparison the N-terminal specific antibody also interacts with the proteolysed form of SNAP-25.

[0031] FIG. 4. Characterization of the binding affinity of BACS antibodies. Multi-well plates were coated with a dilution series of SNAP-25 and binding affinities were measured by ELISA. All four antibodies displayed sigmoidal binding characteristics and possess dissociation constants that range from 0.92 to 1.48 Nm.

[0032] FIG. 5. A drawing of a direct ELISA process using BACS antibody F2691 and commercially available cleavage insensitive antibody (66066).

[0033] FIG. 6. A graph showing that both antibodies (F2691 and 66066) interact with recombinant SNAP-25.

[0034] FIG. 7. A graph showing ELISA response for both F2691 and 66066 antibodies in neuron lysate treated with 0-30 Nm BoNT/A. The signal generated with BACS antibody F2691 shows dose response commensurate with BoNT/A concentration.

[0035] FIG. 8. A drawing of a capture ELISA process using the N-terminal antibody 66066 and the cleavage sensitive antibody F2070.

[0036] FIG. 9. A graph demonstrating that the combination of 66066 and F2070 antibodies avidly binds and detects recombinant SNAP-25.

[0037] FIG. 10. A graph showing the amount of captured full length SNAP-25 from neuron lysate treated with 0-30 Nm BoNT/A.

[0038] FIG. 11. A graph and photograph showing the amount of full length SNAP-25 measured by Western blot analysis as a function of BoNT/A concentration. ELISA signals measured using BACS antibodies (FIGS. 7 & 10) show similar dose-dependent responses.

[0039] FIG. 12. A graph of average fluorescence ratios (intensities at 700/800 nm which corresponds to total SNAP-25/full length SNAP-25) measured by low resolution fluorescence imaging using BACS antibodies plotted against BoNT

A toxin concentration. Data presented in the graph are average values of six replicates, and error bars represent standard errors of the averages.

[0040] FIG. 13. A panel of photographs showing high resolution immunofluorescence analysis using N-terminal antibody SMI-81 and BACS antibody F2070. After a 3 hour exposure to 5 Nm BoNT A, cleaved SNAP-25 is recognized by SMI-81 but not by F2070, resulting in a reduction in fluorescence.

[0041] FIG. 14. A graph demonstrating assay stability validation. Chick motor neurons were harvested and transferred to a 96 well plate. Half of the plated cells were intoxicated with 2 Nm BoNT/A and the other half were incubated with media alone. After a 3 hour incubation period, cells were fixed, stained with N-terminal specific antibody SMI-81, BACS antibody F2070, and their cognate fluorescently labeled secondary antibodies, and analyzed using the low resolution intracellular fluorescence assay (in-cell Western blot analysis). Fluorescence intensity in each channel was calculated for each well, and the ratio of intensities at 700 and 800 nm (corresponding to total SNAP-25/Full length SNAP-25) was plotted against well position (i.e., replicate).

[0042] FIG. 15. A graph depicting the results of a BACS assay performance using a known BoNT/A antagonist. Neurons were harvested, plated, incubated with media containing BoNT/A (0, 0.5 or 2 Nm) or BoNT/A (2Nm) plus antibody, and analyzed by the standardized BACS low resolution fluorescence assay. A staph enterotoxin B (SEB) neutralizing antibody did not affect BoNT/A toxin activity, but a previously characterized neutralizing antibody (4A2-4, i.e., Anti-BoNT/A) efficiently inhibited SNAP-25 cleavage at 2Nm BoNT/A. Data presented in the bar graph are average values of six replicates, and error bars represent standard errors of the averages.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0043] In the following detailed description, for purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the various embodiments of the present invention. It will be apparent, however, that the various embodiments of the present invention may be practiced without these specific details. In other instances, well-known structures and devices are schematically shown in order to simplify the drawing.

[0044] Suitable substrates for the toxin assay include the protein families VAMP (also known as synaptobrevin), SNAP-25,35 and syntaxin. Each of these protein families comprises several isoforms and analogues which are detailed below.

[0045] In mammals, VAMP (or synaptobrevin) has isoforms 1 and 2, and cellubrevin which is found in non-secretory cells. There may be other isoforms in exocrine cells.

[0046] SNAP-25 also has two known isoforms (a and b) and an analogue called SNAP-23.

[0047] Syntaxin has large number of isoforms, divided into groups 1-6. Some of these isoforms have sub-groups.

[0048] Detailed descriptions of the various members of the substrate protein families are given in the following published papers, which are hereby incorporated by reference in their entirety:

VAMP:

[0049] Archer, III, B. T, Ozcelik, T, Jahn, R., Francke, U. and Sudhof, T. C., "Structures and chromosomal localiza-