

BRIEF SUMMARY

[0010] An embodiment is an isolated peptide comprising the amino acid sequence X1 X2 R I D X3 A N Q R A T X4 X5 (SEQ ID NO: 1) wherein each of X1 through X5 is chosen from the following: X1 is K, R, ornithine, or 2, 4-diaminobutanoic acid (dbu); X2 is V, 2-aminobutanoic acid (B), I, L, or T; X3 is Q, A, norvaline (nV), B, or E; X4 is K, R, or dbu; and X5 is M or norleucine (nL); except wherein X1=K, X2=T, X3=E, X4=K, and X5=M; X1=K, X2=T, X3=Q, X4=K, and X5=M; or X1=R, X2=T, X3=Q, X4=R, and X5=M. In an embodiment, SEQ ID NO: 1 is modified with a fluorophore located on one side of a BoNT A cleavage site and a quencher located on the other side of the BoNT A cleavage site.

[0011] In another embodiment, the isolated peptide comprises the amino acid sequence R V R I D A A N Q R A T R M (SEQ ID NO: 2). In yet another embodiment, the isolated peptide is modified with a fluorophore located on one side of a BoNT A cleavage site and a quencher located on the other side of the BoNT A cleavage site. In an embodiment, the fluorophore is located at one terminus and a quencher is located at the other terminus. In an embodiment, DabcylK is located at the N-terminus and S-fluoresceinyl cysteine is located at the C-terminus.

[0012] In another embodiment, the isolated peptide comprises the amino acid sequence R V R I D A A N Q R A T R nL (SEQ ID NO: 3). In an embodiment, the isolated peptide is modified with a fluorophore located on one side of a BoNT A cleavage site and a quencher located on the other side of the BoNT A cleavage site. In another embodiment, the fluorophore is located at one terminus and a quencher is located at the other terminus. In an embodiment, DabcylK is located at the N-terminus and S-fluoresceinyl cysteine is located at the C-terminus.

[0013] An embodiment is a kit to detect the presence of BoNT A in a sample comprising the isolated peptide SEQ ID NO: 1 modified with a fluorophore located on one side of a BoNT A cleavage site and a quencher located on the other side of the BoNT A cleavage site; polymeric beads coated with antibodies specific for BoNT A; and polymeric beads coated with immunoglobulins not specific for BoNT A. In an embodiment, the kit further comprises lyophilized BoNT A Lc. In another embodiment, the kit further comprises a pH-buffering compound in a first container. In yet another embodiment, the pH-buffering compound is selected from the group consisting of sodium hydroxyethylpiperazine sulfonate (HEPES) and sodium phosphate. In still another embodiment, the pH-buffering compound is in dry form. In an embodiment, the kit further comprises bovine serum albumin. In another embodiment, the kit further comprises polysorbate 20. In an embodiment, the polysorbate 20 is added to a final concentration of 0.05-0.10% (v/v). In an embodiment, the kit further comprises a reducing agent in a first container. In an embodiment, the reducing agent is selected from the group consisting of dithiothreitol and tris-(carboxyethyl)-phosphine. In another embodiment, the kit further comprises a zinc salt in a first container. In an embodiment, the zinc salt is selected from the group consisting of zinc chloride and zinc acetate. In yet another embodiment, the kit further comprises purified water in a second container. In still another embodiment, the kit further comprises purified dimethylsulfoxide in a third container. In an embodiment, the lyophilized BoNT A Lc comprises a stabilizing excipient. In another embodiment, the isolated peptide is in dry form.

[0014] An embodiment is a method of detecting the presence of BoNT A in a sample comprising placing the sample in solution in a pH-buffering compound; mixing the sample in the pH-buffering compound with polymeric beads coated with antibodies specific for BoNT A to provide a test assay; mixing the sample in the pH-buffering compound with polymeric beads with immunoglobulins not specific for BoNT A to provide a control assay; incubating the sample with the pH-buffering compound with polymeric beads coated with antibodies specific for BoNT A to provide a test assay; incubating the sample with the pH-buffering compound with polymeric beads with immunoglobulins not specific for BoNT A to provide a control assay; washing the polymeric beads coated with antibodies specific for BoNT A with the pH-buffering compound; washing the polymeric beads without antibodies with the pH-buffering compound; suspending the beads from the test assay in a first container comprising the pH-buffering compound, dithiothreitol, and a zinc salt; suspending the beads from the control assay a second container comprising the pH-buffering compound, dithiothreitol, and a zinc salt; adding isolated peptide SEQ ID NO: 1 that is modified with a fluorophore located on one side of a BoNT A cleavage site and a quencher located on the other side of the BoNT A cleavage site to the test assay and control assay; incubating the isolated peptide with the beads from the test assay; incubating the isolated peptide with the beads from the control assay; separating the beads from the test assay from a test assay solution comprising the isolated peptide; separating the beads from the control assay from a control assay solution comprising the isolated peptide; measuring fluorescence intensity of the test assay solution; measuring fluorescence intensity of the control assay solution; comparing the fluorescence of the test assay solution and the control assay solution; and determining whether BoNT A is present in the sample by whether the fluorescence of the test assay solution is higher than the fluorescence of the control assay solution. In an embodiment, the method further comprises adding bovine serum albumin to the pH buffering compound. In another embodiment, the method further comprises adding polysorbate 20 present to the pH buffering compound. In an embodiment, the polysorbate 20 is added to a final concentration of 0.05-0.10%.

[0015] An embodiment is a method of determining the concentration of BoNT A in a test sample comprising placing the sample in solution in a pH-buffering compound; mixing the sample in the pH-buffering compound with polymeric beads coated with antibodies specific for BoNT A to provide a test assay; mixing the sample in the pH-buffering compound with polymeric beads with immunoglobulins not specific for BoNT A to provide a control assay; incubating the sample with the pH-buffering compound with polymeric beads coated with antibodies specific for BoNT A to provide a test assay; incubating the sample with the pH-buffering compound with polymeric beads with immunoglobulins not specific for BoNT A to provide a control assay; washing the polymeric beads coated with antibodies specific for BoNT A with the pH-buffering compound; washing the polymeric beads without antibodies with the pH-buffering compound; suspending the beads from the test assay in a first container comprising the pH-buffering compound, dithiothreitol, and a zinc salt; suspending the beads from the control assay a second container comprising the pH-buffering compound, dithiothreitol, and a zinc salt; adding the isolated peptide SEQ ID NO: 1 that is modified with a fluorophore located on one