



## Method and Compositions for Detecting Botulinum Neurotoxin

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**The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a method for the sensitive detection of botulinum neurotoxin both *in vitro* and in living cells.**

### Overview

Botulinum neurotoxins produced by *Clostridium botulinum* are the most potent toxins known. Because of the threat of bio-terrorism, sensitive methods for detecting botulinum neurotoxin are needed; however, current detection methods are expensive, time-consuming and complicated.

### The Invention

UW-Madison researchers have developed a fluorescence resonance energy transfer (FRET) method for the sensitive detection of botulinum neurotoxin. The assay uses two fluorescent proteins, such as cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), which are linked together by a molecule that can be recognized and cleaved by botulinum neurotoxin. The emission spectrum of CFP partially overlaps with that of YFP. As a result, when CFP and YFP are very close together, excitation of CFP results in FRET-YFP emission and partial quenching of CFP emission. When botulinum neurotoxin cleaves the linker molecule separating these two fluorescent proteins, FRET is eliminated, i.e., excitation of CFP no longer results in YFP emission and partial quenching of CFP emission.

To detect botulinum neurotoxin, a sample is exposed to the CFP and YFP construct. The FRET signals are measured and compared before and after exposure, with a decrease in FRET after exposure indicating the toxin's presence. This method is useful for detecting botulinum toxin both *in vitro* and in living cells.

### Applications

- Provides an *in vitro* sensor for botulinum neurotoxin
- Assaying the entry of the toxin into living cells
- Cell-based screening of botulinum neurotoxin inhibitors

### Key Benefits

- Highly sensitive: detects botulinum neurotoxin at the picomolar level
- Amenable to high throughput automated screening
- Valuable in the defense industry as well as for civilian bioterrorism prevention

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