



## Assays for Botulinum Neurotoxin Detection and Quantification

BioSentinel offers a suite of products for your botulinum neurotoxin (BoNT) detection needs. Whether you need to determine the specific activity of a BoNT preparation, characterize a BoNT inhibitor, or quantify the amount of BoNT contained in a blood sample, BioSentinel has an assay solution for you. Our products offer mouse assay sensitivity levels in high throughput formats with the ability to detect multiple BoNT serotypes in a wide range of complex matrices.

The BoTest™ and BoTest™ Matrix assays offer real solutions for your botulinum neurotoxin detection needs.

- Up to a 300-fold increase in sensitivity to BoNT compared to other commercially available assays
- Ratiometric, mix and read, FRET-based format more robust than existing intensity-based assays
- Use native BoNT substrates for improved enzyme binding and sensitivity
- Ability to detect BoNTs in complex matrices
- Reagent consistency and reliability

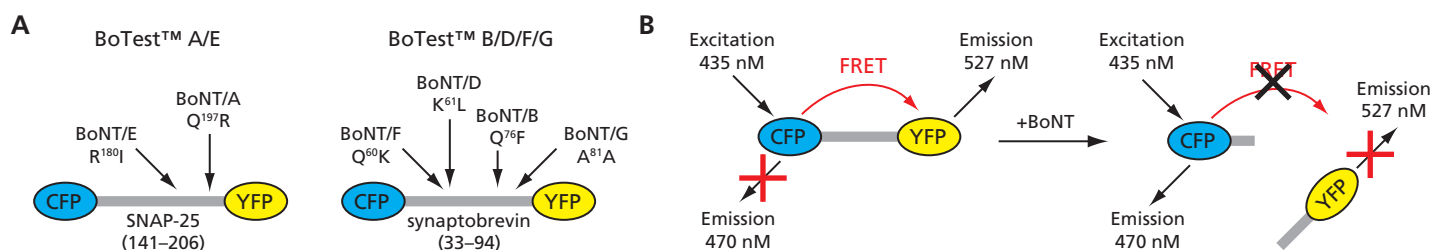
# BoTest™ A/E and BoTest™ B/D/F/G

## Botulinum Neurotoxin Detection Assays

for drug discovery, basic research, and real-time detection

BioSentinel's BoTest™ Botulinum Neurotoxin (BoNT) Detection Assays offer the most sensitive system available for the routine detection of BoNT serotypes A and E (BoTest™ A/E), and serotypes B, D, F, G (BoTest™ B/D/F/G). The BoTest™ assays measure the ability of BoNT to proteolytically cleave the natural BoNT substrates – SNAP25 or VAMP2 – in a sensitive, FRET-based, mix and read format and using most standard fluorescent plate readers (Figure 1). The substrates used in the assay encompass both the exosite binding sites and cleavage site of BoNT, resulting in very high BoNT affinity for the substrate and picomolar detection sensitivities within a few minutes to a few hours. The FRET-based nature of the assays allows for real-time detection of BoNT proteolytic activity enabling the determination of kinetic constants and the determination of enzymatic activity.

Figure 1. Composition of the BoTest™ reporters.



### DETECTION OF SIX BoNT SEROTYPES

The BoTest™ assays can detect six of seven serotypes of BoNT in real-time and endpoint modes. Both BoNT serotype A and trypsinized serotype E are detected with the BoTest™ A/E assay (Figure 2). BoNT serotypes B, D, F, and G (trypsinized) are detected by the BoTest™ B/D/F/G assay (Figure 3).

Figure 2. Detection of BoNT/A and E using the BoTest™ A/E BoNT Detection Kit.

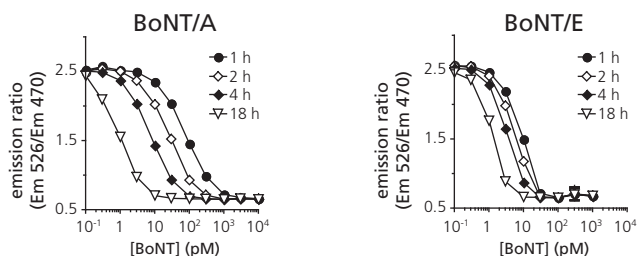
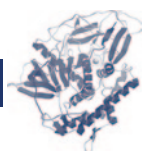
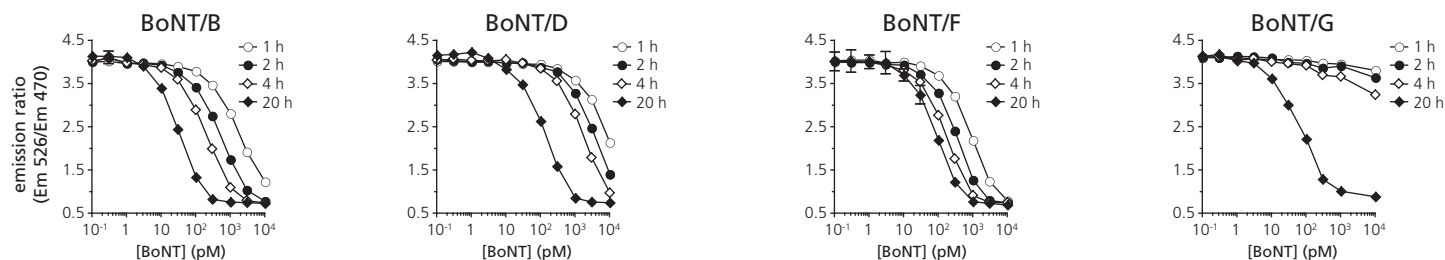


Figure 3. Detection of BoNT/B, D, F, and G using the BoTest™ B/D/F/G BoNT Detection Kit.



## PICOMOLAR TO FEMTOMOLAR SENSITIVITY

Depending on the BoNT serotype and the assay time, picomolar to femtomolar detection limits are possible with the BoTest™ assay (Table 1). The BoTest™ assays can be run at temperatures between room temperature and 37 °C allowing end-users to tailor the assays to their particular needs. The BoTest™ assays are the most sensitive and flexible BoNT detection assays on the market.

**Table 1. Limits of detection for the BoTest™ reporters at varying times.**

time (h)	BoTest™ A/E		BoTest™ B/D/F/G			
	BoNT/A	BoNT/E <sub>tryp</sub>	BoNT/B	BoNT/D	BoNT/F	BoNT/G <sub>tryp</sub>
4	0.3 pM	1 pM	30 pM	300 pM	30 pM	300 pM
20	0.3 pM	0.3 pM	10 pM	100 pM	3 pM	30 pM

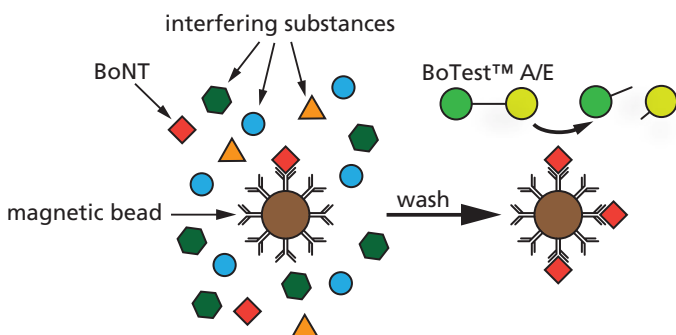
## BoTest™ Matrix A and E Botulinum Neurotoxin Detection Assays for detection and quantification of BoNT in complex and dilute samples

The BoTest™ Matrix A and E BoNT Detection Assays combine the sensitivity and convenience of the BoTest™ assays with the power of immunoprecipitation in order to measure BoNT activity in complex samples. The Matrix A and Matrix E beads used in these kits consist of magnetic beads conjugated to serotype-specific antibodies directed against BoNT/A and E, respectively. The Matrix beads allow for the binding, concentration, and isolation of BoNT/A and E from complex matrices. The captured BoNT can then be quantified using the BoTest™ A/E reporter.

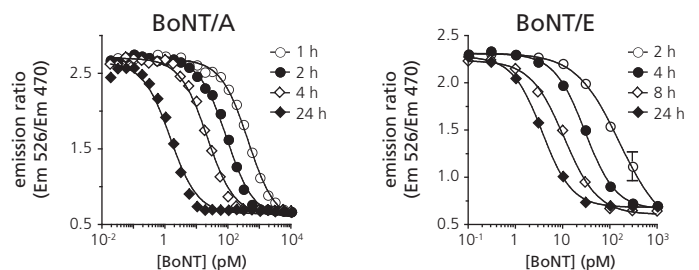
### IMMUNOPRECIPITATION AND QUANTIFICATION OF BoNT/A AND E

The BoTest™ Matrix A and E assays (Figure 4) can isolate and detect picomolar quantities of BoNT/A or E in as little as 3 hours or femtomolar quantities in less than 24 hours. Like the BoTest™ assays, the sensitivity of the BoTest™ Matrix assay can be adjusted with incubation time (Figure 5).

**Figure 4. Illustration of the BoTest™ Matrix Assay.**



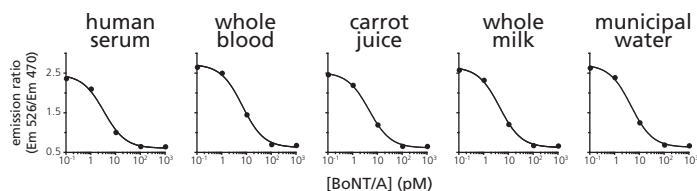
**Figure 5. Detection of BoNT/A and E using the BoTest™ Matrix A and E BoNT Detection Kits.**



## DETECTION OF BoNT/A IN COMPLEX MATRICES

The inclusion of the Matrix beads allows for the isolation of BoNT and the removal of substances that might otherwise interfere with BoNT activity in vitro. The BoTest™ Matrix assays are compatible with a range of complex matrices (Figure 6) including pharmaceutical BoNT/A preparations (Figure 7).

**Figure 6.** Detection of BoNT/A in complex matrices.



## DETECTION OF BoNT/E IN FIELD SAMPLES

The BoTest™ Matrix assays are applicable to “real world” samples, not just purified pharmaceutical products or research preparations. For instance, the BoTest™ Matrix E assay was used to quantify the amount of BoNT/E contained in avian (bird) blood samples collected during a botulism outbreak (Figure 8).

## ORDERING INFORMATION

To order, visit [www.biosentinelpharma.com](http://www.biosentinelpharma.com) or call toll free 1-866-807-0324. BoTest™ and BoTest™ Matrix Assays are available in convenient, pre-packed sizes; contact customer service for custom quantities.

Description	Size	Part number	Price*
BoTest™ A/E BoNT Detection Kit	200 assays	A1004	\$ 426/875
BoTest™ B/D/F/G BoNT Detection Kit	200 assays	A1009	\$ 426/875
BoTest™ Matrix A BoNT Detection Kit	200 assays	A1015	\$1177/2075
BoTest™ Matrix E BoNT Detection Kit	200 assays	A1019‡	\$1177/2075

\* Prices for US Government laboratories/commercial laboratories. Other parties interested in using the BoTest™ or BoTest™ Matrix assays should contact BioSentinel Customer Service for more price information.

‡ BoTest Matrix E available July 2011.

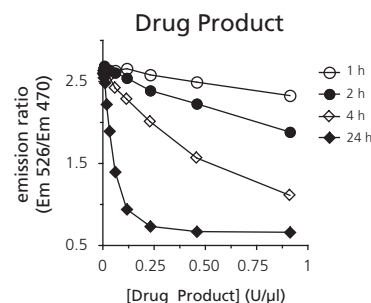
Note: Botulinum toxin is not supplied with these kits.

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**Figure 7.** Quantification of the BoNT/A proteolytic activity contained in drug product.



**Figure 8.** BoNT/E quantification of avian samples.

