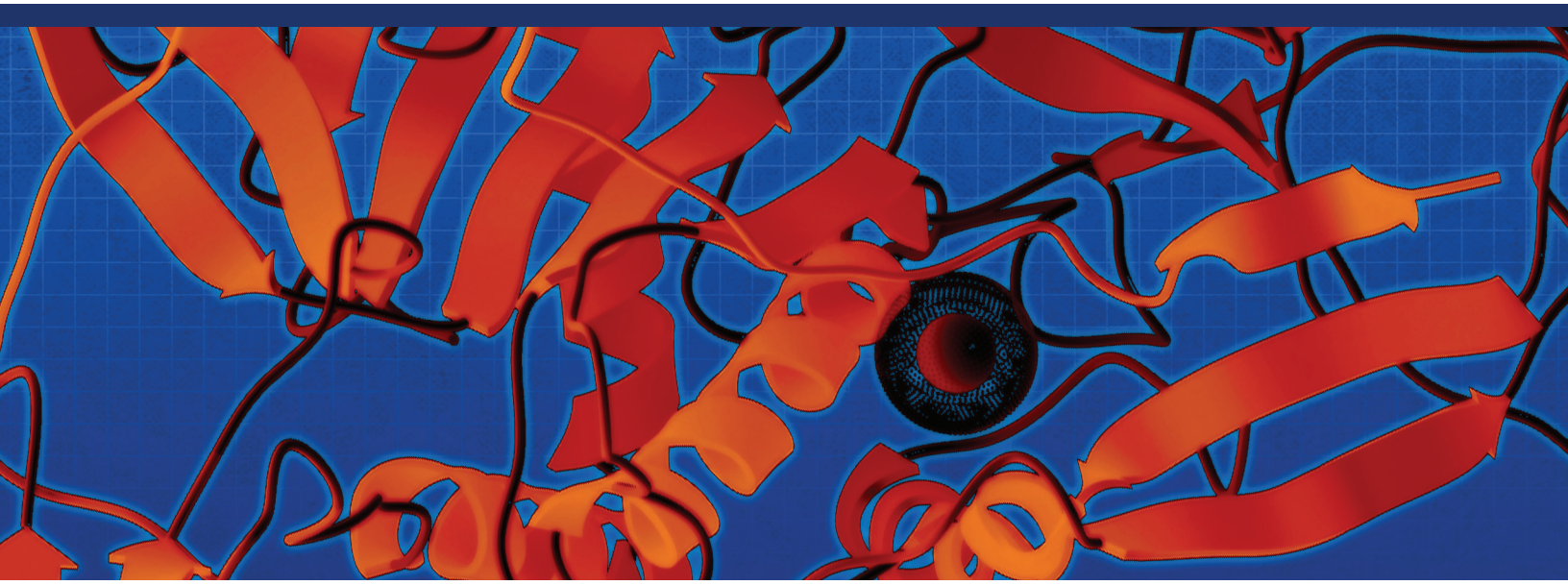
BioSentinel



Assays for Botulinum Neurotoxin Detection and Quantification

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

The BoTest® and BoTest® Matrix assays offer real solutions for your BoNT detection needs.

- Up to a 300-fold increase in BoNT sensitivity compared to other commercially available assays
- Ratiometric, mix-and-read, FRET-based format that is more robust than existing intensity-based assays
- Improved enzyme binding and sensitivity using native BoNT substrates Detect BoNTs in complex matrices
- Reagent consistency and reliability

BioSentinel • 505 S. Rosa Road • Suite 105 • Madison, WI 53719-1267

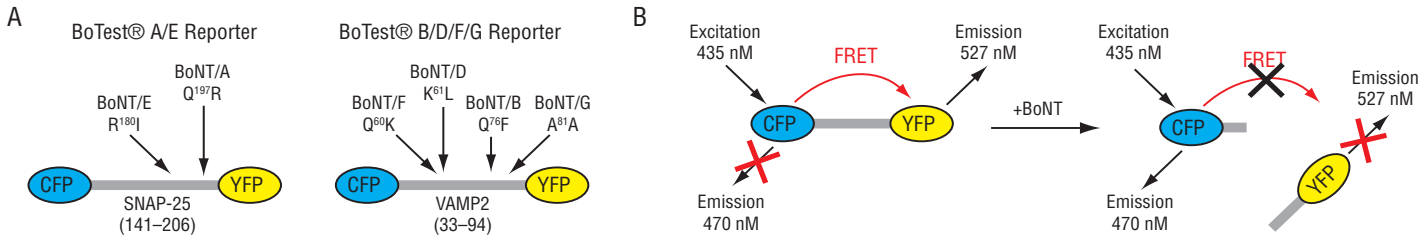
BoTest® A/E and BoTest® B/D/F/G

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 BoNTs to proteolytically cleave their natural BoNT substrates — SNAP25 or VAMP2 — in a sensitive, FRET-based, mix-and-read format using most standard fluorescence plate readers (**Figure 1**). The substrates used in the assay encompass both the BoNT exosite binding and cleavage sites, resulting in very high BoNT substrate affinity and picomolar detection sensitivities within a few minutes to a few hours. The FRET-based nature of the assays allows real-time detection of BoNT proteolytic activity and enables determination of kinetic constants and 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.

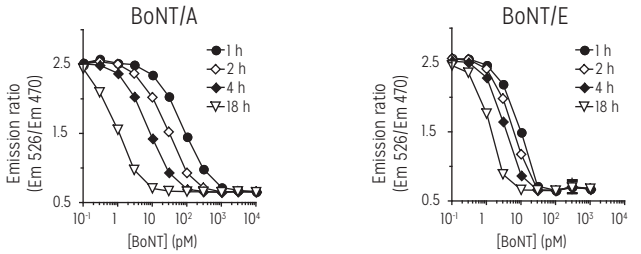
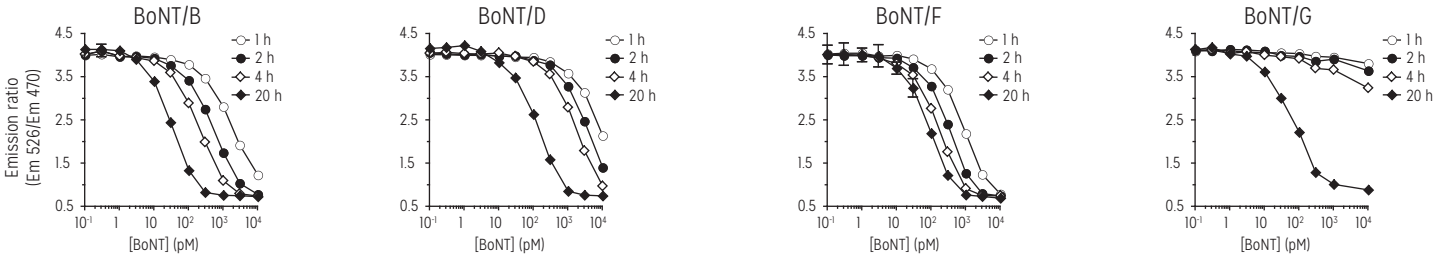


Figure 3. Detection of BoNT/B, D, F, and G using the BoTest® B/D/F/G BoNT Detection Kit.



PICOMOLAR TO FEMTOMOLAR SENSITIVITY

Depending on the BoNT serotype and the assay time, picomolar to femtomolar detection limits with small sample sizes (e.g., 100 µl) are possible with the BoTest® assays (**Table 1**). The BoTest® assays can be run at temperatures between room temperature and 37 °C so end-users can 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_{tryp}	BoNT/B	BoNT/D	BoNT/F	BoNT/G_{tryp}
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, B, E, and F

Botulinum Neurotoxin Detection Assays

for activity detection and quantification of BoNT in complex and dilute samples

The BoTest® Matrix A, B, E, and F BoNT Detection Assays combine the sensitivity and convenience of the BoTest® assays with the power of immunoprecipitation to measure BoNT activity in complex samples. The Matrix beads are magnetic beads conjugated to serotype-specific antibodies directed against BoNT/A, B, E, and F, and allow for binding, concentrating, and isolating the corresponding BoNT serotype from complex matrices. The captured BoNT can then be quantified using the BoTest® A/E or the BoTest® B/D/F/G reporter.

IMMUNOPRECIPITATION AND QUANTIFICATION OF BONT/A AND E

The BoTest® Matrix assays (**Figure 4**) can isolate and detect picomolar quantities of BoNT 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**). Additional sensitivity gains are possible by using larger sample volumes from 200 l to 15 ml.

Figure 4. Illustration of the BoTest® Matrix Assay.

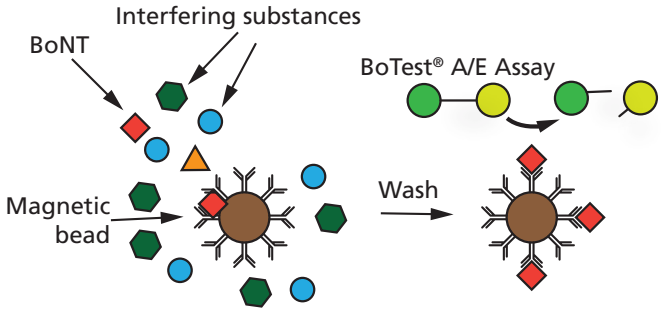
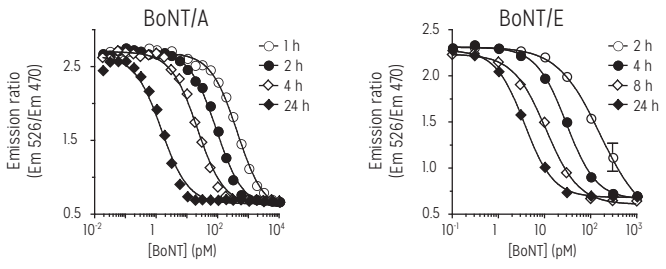


Figure 5. Detection of BoNT/A and E using the BoTest® Matrix A and E BoNT Detection Kits.



DETECTION OF BoNT/A IN COMPLEX MATRICES

The Matrix beads enable isolation of BoNT and removal of substances that might otherwise interfere with BoNT activity in vitro. Thus, the BoTest® Matrix assays are compatible with a range of complex matrices (**Figure 6**) including pharmaceutical BoNT/A preparations (**Figure 7**), foods, and serum samples.

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

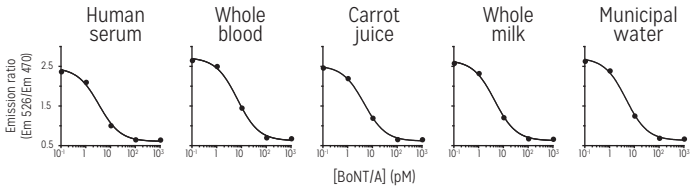
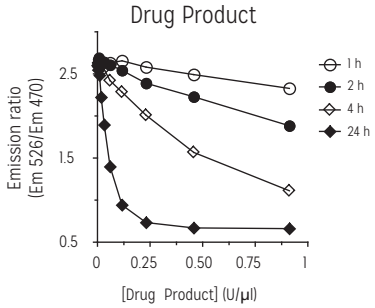


Figure 7. Quantification of the BoNT/A proteolytic activity contained in drug product..



BoLISA® A, B, C, and E

Botulinum Neurotoxin Sandwich ELISA Detection Kits

for detection and quantification of BoNT using a well-known format

The BoLISA® Botulinum Neurotoxin Detection Assays are highly serotype-specific sandwich ELISAs for detecting femto- to pico-molar quantities of BoNT in complex and dilute matrices. The BoLISA® capture antibodies are compatible with standard ELISA plates, while the biotinylated detection antibody is compatible with a wide range of colorimetric, fluorometric, or luminescence detection systems. **Figure 8** shows serotype-specific detection of BoNT/A (Figure 8, **Panel A)**, B (Figure 8, **Panel B**), C (Figure 8, **Panel C**), and E (Figure 8, **Panel D**) in dilute matrices. Figure 9 shows detection of BoNT/A in complex food matrices.

Figure 8. Serotype-specific detection of BoNT/A, B, C, and E (Panels A, B, C, and D, respectively) in phosphate-buffered saline (PBS).

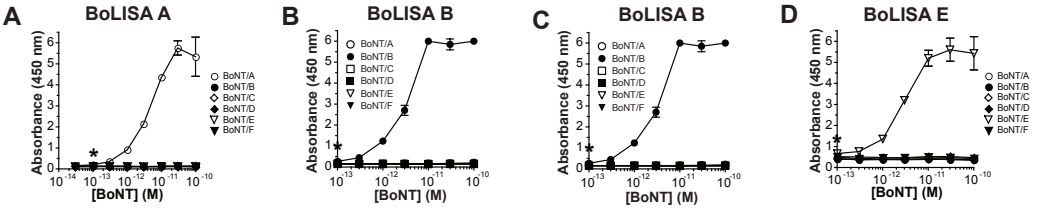
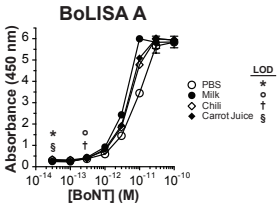


Figure 9. Serotype-specific detection of BoNT/A in complex food samples.



Note: Botulinum toxin is not supplied with these kits.
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