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>
<thead>
<tr>
<th>time (h)</th>
<th>BoTest® A/E</th>
<th>BoTest® B/D/F/G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BoNT/A</td>
<td>BoNT/E</td>
</tr>
<tr>
<td>4</td>
<td>0.3 pM</td>
<td>1 pM</td>
</tr>
<tr>
<td>20</td>
<td>0.3 pM</td>
<td>0.3 pM</td>
</tr>
</tbody>
</table>

**BoTest® Matrix A, B and F (Matrix E coming soon)**

**Botulinum Neurotoxin Detection Assays**

for detection and quantification of BoNT in complex and dilute samples

The BoTest® Matrix A, B and F 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, B and F from complex matrices. The captured BoNT can then be quantified using the BoTest® A/E reporter or B/D/F/G 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).
**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.**

**BoLISA™ A BOTULINUM NEUROTOXIN DETECTION ASSAY (BoLISA C AND E KITS COMING SOON)**

BoLISA™ A Botulinum Neurotoxin Detection Assay is a highly serotype-specific sandwich ELISA-based detection assay for detecting femtomolar quantities of BoNT/A in complex and dilute matrices. The BoLISA capture antibody is compatible with a variety of plate types, while the biotinylated detection antibody is compatible with a wide variety of colorimetric, fluorometric, or luminescence detection systems.

**Figure 8. Serotype-specific detection of BoNT/A in phosphate-buffered saline (Panels A) and in complex food samples (Panel B).**

![BoNT/A detection graph](image)

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

Note: Botulinum toxin is not supplied with these kits. BoTest® is a registered trademark of BioSentinel, Inc. CFP and YFP are used under license from Life Technologies and GE. Literature Part No. L1008 (Revised September 2013)