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Product #190B  
Lot #19050A2  
Release Date: Sept 2019

CERTIFICATE OF ANALYSIS  
TETANUS TOXIN  
from *Clostridium tetani*  
Lot #19050A2

**New Quantitation**

**Contents**

Each vial contains 100 µg of tetanus toxin. When reconstituted to 400 µL with water, the buffer is 20 mM HEPES, pH 7.4 with 1.25% lactose. **Handle the product gently; do not vortex.**

**Concentration**

The protein concentration was determined by absorbance at 280 nm using an extinction coefficient of 1.26 for a 1 mg/ml solution. This value is calculated by ProtParam<sup>1</sup> using an algorithm based on the Edelhoch<sup>2</sup> method with modifications described in Pace et al<sup>3</sup>.

NOTE: The concentration for previous lots of Product #190B was determined by a modification of the method of Bradford<sup>4</sup> using ovalbumin as the standard.

**Purity**

When examined on 4 – 12% SDS-PAGE, this protein migrates as a single major band with an apparent molecular weight of approximately 150,000 daltons. In the presence of a reducing agent, the preparation migrates as two bands with apparent molecular weights of 100,000 and 50,000 daltons. Densitometric analysis estimates the purity as >80%.

The endotoxin content, determined using a kinetic chromogenic LAL assay, is 2 EU/mg.

**Activity**

Binding activity to G<sub>T1b</sub> ganglioside in a hemagglutination assay is also assessed.<sup>5</sup> Hemagglutination is evident at 6.25 µg/ml Tetanus Toxin.

The tetanus toxin has been tested for enzymatic activity in a FRET based assay. Digestion of the FRET substrate was detected within 3 hours when using 200 nM tetanus toxin with 20 µM VAMPtide (Product #540).

**Toxicity**

Tetanus toxin is one of the most deadly toxins known to man. Even small amounts of tetanus toxin can pose a serious threat to an unvaccinated user. Consult the SDS for further information.

**Packaging/Storage**

This preparation is provided as a lyophilized powder that has been stoppered under vacuum. Prior to reconstitution, it should be stored at 2 – 8°C.

### **Handling**

Good laboratory technique should be employed in the safe handling of this product. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses. Nitrile gloves are recommended for use when handling lyophilized material.

This product is intended for research purposes by qualified personnel. It is not intended for use in humans or as a diagnostic agent. List Biological Laboratories, Inc., is not liable for any damages resulting from the misuse or handling of this product.

**FOR RESEARCH PURPOSES ONLY. NOT FOR HUMAN USE.**

### **References**

1. [www.expasy.ch/tools/protparam-doc.html](http://www.expasy.ch/tools/protparam-doc.html)
2. Edelhoch, H. (1967) *Biochemistry*, **6**, 1948-1954.
3. Pace, C.N., Vajdos, F., Fee, L., Grimsley, G. and Gray, T. (1995) *Protein Sci.* **4**, 2411-2423.
4. Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248 – 254.
5. Tayot, J.-L., Holmgren, J., Svennerholm L., Lindblad, M. and Tardy, M. (1981) *Eur. J. Biochem.* **113**, 249 – 258.

### **Recommendations**

List Labs recommends that any person who may potentially become exposed to this Toxin should be currently vaccinated with tetanus toxoid.

The Toxin should be controlled and maintained in full compliance with biosafety level 2 or 3 standards, whichever is required based on customer's utilization. Such standards are described in "Biosafety in Microbiology and Biomedical Laboratories" HHS Publication No. (NIH) 88-8395, 2<sup>nd</sup> edition, May 1988 and as the same may be amended or superseded.

Personnel who come into contact with the Toxin shall be fully trained and qualified in its handling and use.

QA/QC: KPD Date: 01/29/2021