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CERTIFICATE OF ANALYSIS  
TETANUS TOXIN  
from *Clostridium tetani*  
Lot #19047A1

### Contents

Each vial, when reconstituted with 100 µl water, contains 25 µg of Tetanus Toxin in 20 mM HEPES, pH 7.4 with 1.25% lactose. **Handle the product gently; do not vortex.**

### Concentration

Protein concentration was determined by a modification of the method of Bradford<sup>1</sup> using ovalbumin as the standard.

### Assay Results

When examined on 4 – 12% SDS-polyacrylamide gels, 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 >90%.

This Tetanus Toxin has been tested for enzymatic activity in an endopeptidase assay. Cleavage of 25% of 5 µM GST-Synaptobrevin (Product #510A) was detected in a gel based assay after incubation with 20 nM Tetanus Toxin for one hour. The reaction was performed at 37°C in 0.02 M Tris-HCl, pH 8.0 with 0.05 M Sodium Chloride.

Binding activity to G<sub>T1b</sub> ganglioside in a hemagglutination assay is also assessed.<sup>2</sup> Hemagglutination is evident at 12.5 µg/ml Tetanus Toxin.

The endotoxin content, determined using a kinetic chromogenic LAL assay, is approximately 10 EU/mg.

### 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 MSDS for further information.

### Packaging/Storage

Tetanus Toxin is supplied as lyophilized powder, sealed under vacuum. Store at 2 – 8°C prior to and after reconstitution.

(continued)

## Handling

Good laboratory technique should be employed in the safe handling of this product. This involves observing the following practices:

1. Persons handling this product and contaminated glassware should consult the current version of the Biosafety in Microbiological and Biomedical Laboratories, BMBL.<sup>3</sup>
2. This product is to be used by skilled personnel under the direction of a principal investigator in an appropriate laboratory.
3. Wear appropriate laboratory attire including lab coat, gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material.
4. Because this product is stoppered under vacuum, it is recommended to reconstitute the contents using a syringe in a biological safety cabinet. Never work with the product in the powdered form. Always reconstitute it first.
5. Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body which comes into contact with the product.
6. Avoid accidental autoinoculation by exercising extreme care when handling in conjunction with any injection device.
7. 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. Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248 – 254.
2. Tayot, J.-L., Holmgren, J., Svennerholm L., Lindblad, M. and Tardy, M. (1981) *Eur. J. Biochem.* **113**, 249 – 258.
3. Biosafety in Microbiological and Biomedical Laboratories. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health.

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