



Animal and Plant  
Health Inspection  
Service

Veterinary Services

Center for Veterinary  
Biologics

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## **Clostridium tetani Toxin - Lot ID IRP 648**

**Document Number:** CVB-DAT-0142

**Revision:** 02

**Previous Number:** BB DAT1045.04

**Vault:** CVB-Released

**Section/Area:** CVB-DAT

**Effective Date:** 01 Mar 2021

**Notes:** *Strain or Source: N/A, Fill date: May 25, 2017*

**United States Department of Agriculture  
Animal and Plant Health Inspection Service  
Center for Veterinary Biologics  
P. O. Box 844  
Ames, IA 50010**

1. **Reagent Name:** *Clostridium tetani* Toxin
2. **Strain or Source:** Not applicable
3. **Lot Number:** IRP 648
4. **Fill Date:** May 25, 2017
5. **Expiration Date:** January 31, 2022

Note: This reagent lot will no longer be monitored by the Center for Veterinary Biologics (CVB). Use for serial release testing after the expiration date must be supported by data that has been approved by the CVB per CVB Notice 17-15.

**Precautions: Accidental parenteral inoculation and ingestion of the toxin are the primary hazards associated with this reagent. It is uncertain if tetanus toxin can be absorbed through mucous membranes; consequently, the hazards associated with aerosols and droplets remain unclear. The administration of an adult diphtheria-tetanus toxoid at 10-year intervals reduces the risk of toxin exposures to laboratory personnel and is highly recommended.**

6. **Intended Use:** Use IRP 648 to coat microtiter plates for the indirect enzyme-linked immunosorbent assay (ELISA) as described in **SAM 217**, and for the comparative toxin-antitoxin neutralization test in guinea pigs as described in **SAM 206**.

7. **Instructions for Use:**

**9CFR 113.217 Tetanus Toxoid:** To conduct ELISA, coat each well of the microtiter plate with 100 µL of IRP 648 diluted 1:12 by adding 1 mL IRP 648 + 11 mL antigen coating buffer (ACB) or 2 mL IRP 648 + 22 mL ACB (for two plates) in antigen coating buffer.

**9CFR 113.451 Tetanus Antitoxin:** To conduct comparative toxin-antitoxin neutralization tests in guinea pigs at the 0.10 Antitoxin Unit per mL level, dilute IRP 648 1:825, in 1/15 M phosphate buffered saline, pH 7.4, with 0.2% gelatin (PBS w/gelatin) by adding 1 mL IRP 648 + 99 mL PBS w/ gelatin (1:100); 2 mL (1:100) + 14.5 mL PBS w/ gelatin.

8. **Test of Reagent:** *Determination of the test dose of toxin* – Titrations were performed to determine the optimum toxin concentration for adsorption to 96-well microtiter plates.

*Sterility Test* - The toxin was tested for sterility and found to be free of viable bacteria and fungi according to 9 CFR 113.26.

**9. Container Size, Type, Weight, or Volume:** 4.0-mL screw-cap vials containing 2.3 mL of toxin.

**10. Storage Conditions:** Store IRP 648 at -70°C or lower.

**11. CVB Technical Contact:** Bacteriology Section, Center for Veterinary Biologics, (515) 337-7020 or FAX (515) 337-7673.

**12. Origin and Passage History:** *Clostridium tetani* culture 7010 obtained from Burns Bio-tech was used to produce IRP 648. The history of the culture prior to being sent to the Center for Veterinary Biologics is unknown.

**13. Method of Preparation:** The culture was grown in a 14-liter fermentor vessel containing modified Mueller and Miller medium. The culture was incubated at 35°- 36°C for 110-120 hours. The culture was centrifuged at 10,000 x g in a rotor chamber temperature of 2°- 7°C for 45 minutes. The supernatant was passed through a 0.2-µm Nalgene™ Rapid Flow™ vacuum PES filter. The filtrate was concentrated using a Millipore pellicon cassette system containing a 10,000 NMWL PTGC00005 filter. The material retained by the filter was passed through a sterile 0.2-µm Nalgene™ Rapid Flow™ vacuum PES filter.

**14. Other:** None

Reagent orders and feedback should be sent *including phone number* to the following email address: [VS.DB.CVB.Reagent.Requests@usda.gov](mailto:VS.DB.CVB.Reagent.Requests@usda.gov)

Reagent orders forms (APHIS Form 2018) can be found on the CVB website.