

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 664
4. **Fill Date:** October 31, 2018
5. **Expiration Date:** February 28, 2029

**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 664 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 the ELISA, coat each well of the microtiter plate with 100  $\mu$ L of IRP 664 diluted 1:12 by adding 1 mL IRP 664 + 11 mL antigen coating buffer (ACB) or 2 mL IRP 664 + 22 mL ACB (for two plates).

**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 664 to 1:625 in 1/15 M phosphate buffered saline, pH 7.4, with 0.2% gelatin (PBS w/ gelatin) by adding 1 mL IRP 664 + 9 mL PBS w/ gelatin (1:10); 2 mL (1:10) + 18 mL PBS w/ gelatin; 4 mL (1:100) + 21 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 title 9, *Code of Federal Regulations* (9 CFR), part 113.26.

9. **Container Size, Type, Weight, or Volume:** 4-mL glass screw-cap vials containing 2.3 mL of toxin.
10. **Storage Conditions:** Store IRP 664 at -70°C or lower.
11. **CVB Technical Contact:** Bacteriology Section, Center for Veterinary Biologics, (515) 337-6100.
12. **Origin and Passage History:** Clostridium tetani culture 7010 obtained from Burns Biotech was used to produce IRP 664. 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 10-liter fermentor vessels containing Modified Mueller & Miller Medium. The culture incubated at 35°-36°C for 110-120 hours, harvested, then centrifuged at 10,000 x g in a rotor chamber at 2°-7°C for 45 minutes. The supernatant was passed through a Pall VacuCap® 90 PF filter unit containing 0.8/0.2-µm Supor® membranes. The filtrate was concentrated using a Millipore pellicon cassette system containing a 10,000 NMWL PTGC00005 filter. The concentrated material retained by the filter was passed through a Pall VacuCap® 90 PF filter unit containing 0.8/0.2-µm Supor® membranes.
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.