

**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 sordellii* Toxin
2. **Strain or Source:** Not Applicable
3. **Lot Number:** IRP 604
4. **Fill Date:** September 1, 2011
5. **Expiration Date:** No expiration date has been assigned to this product because *C. sordellii* toxin has demonstrated over time to be very stable if properly stored. This reagent will be routinely monitored by the Center for Veterinary Biologics.

Precautions: This reagent does not present a hazard to laboratory personnel who manipulate the serum provided sound fundamental laboratory practices are followed.

6. **Intended Use:** IRP 604 serves as the standard toxin when conducting *C. sordellii* toxin-neutralization (TN) tests in mice.
7. **Instructions for Use:** *C. sordellii* toxin IRP 604 diluted 1:19 is considered the standard toxin dilution when conducting TN tests in mice as outlined in the Code of Federal Regulations, Title 9 (9 CFR), Part 113.109. The standard toxin dilution is prepared by adding 1.0 mL of well mixed IRP 604 to 18 mL of sterile peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). A volume of 0.5 mL of the toxin diluted 1:19 and 0.5 mL of diluent represents 1.0 L_o toxin dose. A volume of 0.8 mL of the toxin diluted 1:19 and 0.2 mL of diluent represents 1.0 L₊ dose.
8. **Test of Reagent:** *Determination of test dose of toxin* – The L_o and L₊ doses were established by injecting 16 to 20 gram mice intravenously with 0.2 mL of varying amounts of IRP 604 combined with 1.0 mL of *C. sordellii* antitoxin IRP 501 containing 1.0 antitoxin unit per mL (AU/mL). The L_o and L₊ doses were confirmed by injecting 16 to 20 gram mice intravenously with 0.2 mL of varying amounts of IRP 604 combined with 1.0 mL of International Antitoxin containing 1.0 IU/mL.

The L_o dose for the *C. sordellii* TN test is the largest quantity of toxin which can be mixed with 1.0 unit of antitoxin and not cause death in injected mice within 72 hours. The L₊ dose for the *C. sordellii* TN test is the smallest amount of toxin which can be mixed with 1.0 unit of antitoxin and cause death in at least 80% of injected mice within 72 hours.

Determination of LD₅₀ – White Swiss mice weighing 16-20 g were injected intravenously with 0.2 mL of IRP 604 diluted in peptone diluent. The toxin was found to contain 10⁴ lethal dose fifty (LD₅₀) per 0.2 mL.

Sterility test – The toxin was tested for sterility and found to be free of viable bacteria and fungi according to procedures outlined in 9 CFR 113.26.

9. Container Size, Type, Weight, or Volume: 4-mL glass vials containing 1.3 mL of toxin

10. Storage Conditions: Store at -50° to -90°C.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6165 or FAX (515) 337-7673

12. Origin and Passage History: *C. sordellii* culture No. 7502, used to produce IRP 604, was obtained September 16, 1968 from Montana State University, Bozeman, Montana. The number of passages is unknown.

13. Method of Preparation: Culture 7502 was grown in dialysis membranes with a molecular weight cutoff range from 12,000 to 14,000 daltons. The membranes were filled with 0.15 M phosphate buffered saline, pH 7.4, and suspended in 1-liter trypsinizing flasks containing media consisting of Brain Heart Infusion Broth. Actively growing culture was aseptically added to the inside of the dialysis membranes and incubated at 35°C for 24 hours in an anaerobic glove box containing 85% nitrogen (N), 10% hydrogen (H), and 5% carbon dioxide (CO). The culture was centrifuged at 10,000 x g for 60 minutes. The culture supernatant was passed through a sterile Corning 150-mL bottle top filter containing a 0.22-µm membrane.

14. Other: None

Reagent orders and feedback should be sent *including phone number* to the following email address: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

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