

**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 497
4. **Fill Date:** April 24, 2003
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 497 serves as the standard toxin when conducting *C. sordellii* toxin-neutralization (TN) tests in mice.
7. **Instructions for Use:** *C. sordellii* toxin IRP 497 diluted 1:19 is considered the standard toxin dilution when conducting TN tests in mice as outlined in title 9, *Code of Federal Regulations* (9 CFR), section 113.109. The standard toxin dilution is prepared by adding 1.0 mL of well mixed IRP 497 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 L₀ 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 L₀ and L₊ doses were established by injecting 16 to 20 gram mice intravenously with 0.2 mL of varying amounts of IRP 497 combined with 1.0 mL of *C. sordellii* antitoxin IRP 333 containing 1.0 antitoxin unit per mL (AU/mL). The L₀ and L₊ doses were confirmed by injecting 16 to 20 gram mice intravenously with 0.2 mL of varying amounts of IRP 497 combined with 1.0 mL of International Antitoxin containing 1.0 IU/mL.

The L₀ 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 IRP497 diluted in peptone diluent. The toxin was found to contain 10^{4.5} 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: Five-mL glass vials containing 2.3 mL of toxin.

10. Storage Conditions: Store at -70°± 5°C.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: *C. sordellii* culture No. 7502, used to produce IRP 497, was obtained September 16, 1968 from Montana State University, Bozeman, Montana. The number of passages is unknown.

13. Method of Preparation: Culture no. 7502 was grown in a 14-liter New Brunswick fermentor containing peptone and trypticase media. Actively growing culture was aseptically added to the fermentor and incubated at 35°C for approximately 12 hours. The culture was centrifuged at 10,000 x g for 60 minutes and the supernatant passed through a Millipore filtration unit containing a 0.22-µm membrane. The filtrate was further processed using a Millipore pellicon cassette system containing a high volume ultrafilter. The concentrated toxin was adjusted to pH 6.8 and passed through a Millipore filtration unit containing a 0.22-µm membrane.

This is a new reagent. Please verify the L₀ and L₊ doses at your laboratory prior to use.

14. Other:

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:
http://www.aphis.usda.gov/animalhealth/cvb_forms

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