

**United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
1800 Dayton Avenue
Ames, IA 50010**

1. **Reagent Name:** *Clostridium perfringens* type C (beta) toxin
2. **Strain or Source:** Not applicable.
3. **Lot Number:** IRP 624
4. **Fill Date:** October 29, 2014
5. **Expiration Date:** February 28, 2029

Precautions: This reagent does not present a hazard to laboratory personnel who work with the toxin provided good fundamental laboratory techniques are followed.

6. **Intended Use:** IRP 624 serves as the standard toxin when conducting *C. perfringens* type C toxin-neutralization tests in mice.
7. **Instructions for Use:** IRP 624 diluted 1:75 is considered the standard toxin dilution when conducting toxin-neutralization tests in mice as outlined in title 9, *Code of Federal Regulations* (9 CFR), section 113.111, and 9 CFR 113.454. The standard toxin dilution is prepared by adding 0.5 mL of IRP 624 to 4.5 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The toxin is further diluted to 1:75 by adding 1.0 mL of the 1:10 dilution to 6.5 mL of diluent. A volume of 0.5 mL of the toxin diluted 1:75 and 0.5 mL of diluent is equivalent to 10 L_o doses. A volume of 0.9 mL of toxin diluted 1:75 and 0.1 mL of diluent is equivalent to 10 L₊ doses. *C. perfringens* type C (beta) toxin IRP 624 diluted 1:10 is stable when stored at -60°C or lower.
8. **Test of Reagent:** *Determination of test dose of toxin* - The 10 L_o and 10 L₊ doses were established by injecting mice intravenously with 0.2 mL of a mixture of varying amounts of IRP 624 combined with 10 International Units (IU) of *C. perfringens* beta antitoxin. The 10 L_o dose for *C. perfringens* type C (beta) toxin neutralization test is the largest amount of toxin which can be mixed with 10 IU of beta antitoxin and not cause death in injected mice within 24 hours. The 10 L₊ dose for *C. perfringens* type C (beta) toxin neutralization test is the smallest amount of toxin which can be mixed with 10 IU of beta antitoxin and cause death in at least 80% of injected mice within 24 hours.

Determination of LD₅₀ in mice - Female white Swiss mice weighing 16-20 g were injected intravenously with 0.2 mL of IRP 624 diluted in peptone diluent. The toxin was found to contain 10^{4.3247} mouse lethal dose fifty (LD₅₀) per mL.

Determination of toxin type - The toxin type was confirmed by performing toxin neutralization tests in mice. The mice were injected intravenously with mixtures of IRP 624 and *C. perfringens* type A, B, C, or D antitoxin. All of the mice died within 24 hours except those receiving mixtures containing type B or C antitoxin.

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

9. Container Size, Type, Weight, or Volume: 1.5-mL glass vials containing 0.8 mL of toxin.

10. Storage Conditions: Store at $-70^{\circ}\pm 10^{\circ}\text{C}$.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6100 or FAX (515) 337-7673.

12. Origin and Passage History: *C. perfringens* type C (beta) culture #4414, used to produce IRP 624, was obtained from Coopers Animal Health, Inc., on July 28, 1975. The number of passages is unknown.

13. Method of Preparation: Culture #4414 was grown in a 14-liter New Brunswick fermentor containing media consisting of N-Z case, proteose peptone, and yeast extract. Actively growing culture was aseptically added to the fermentor and incubated at 35°C for approximately 4 hours. The culture was centrifuged at $10,000 \times g$ for 60 minutes. The supernatant was passed through a Millipore filtration unit containing a 0.2- μm membrane. The filtrate was further processed using a Millipore pellicon cassette system containing a high volume ultra-filter. The concentrated toxin was adjusted to pH 6.6 and passed through a sterile Millipore filtration unit containing a 0.22-mm membrane. Sterile glycerol was added to the product at a final concentration of 15% v/v.

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

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