Veterinary Services
Safeguarding Animal Health

Animal and Plant Health Inspection Service

Veterinary Services

Center for Veterinary Biologics

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Clostridium novyi Type B Toxin - Lot ID IRP 636

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Notes: *Strain or Source: N/A, Fill date: 06Oct16*

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 novyi* Type B Toxin

2. Strain or Source: Not applicable

3. Lot Number: IRP 636

4. Fill Date: October 06, 2016

5. Expiration Date: March 30, 2024

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

6. Intended Use: IRP 636 serves as the standard toxin when conducting *C. novyi* alpha toxin-neutralization tests in mice.

7. Instructions for Use: IRP 636 diluted 1:30 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 113.108. The solution of test toxin is prepared by adding 0.5 mL of well mixed IRP 636 to 14.5 mL of sterile peptone diluent (1.0% peptone, 0.25% sodium chloride, (pH 7.2)). A volume of 0.6 mL of the toxin diluted 1:30 and 0.4 mL of diluent is equivalent to 0.1 L_o dose. A volume of 0.9 mL of toxin and 0.1 mL of diluent is equivalent to 0.1 L_+ dose.

8. Test of Reagent:

Determination of test dose of toxin – The 0.1 L_o and 0.1 L_+ doses were established by injecting mice intravenously with 0.2 ml of a mixture of varying amounts of IRP 636 combined with 0.1 International Unit of antitoxin. The 0.1 L_o dose for the *C. novyi* type B toxin neutralization test is the largest amount of toxin which can be mixed with one-tenth unit of Standard Antitoxin and not cause death in injected mice within 72 hours. The 0.1 L_+ dose for the *C. novyi* type B toxin neutralization test is the smallest amount of toxin which can be mixed with one-tenth unit of Standard Antitoxin and cause death in at least 80% of injected mice within 72 hours.

Determination of toxin type – Toxicity studies in mice demonstrated that the lethal effect of *C. novyi* type B toxin IRP 636 was neutralized by *C. novyi* type A (alpha) antitoxin. Test results showed that IRP 636 contains no detectable beta toxin.

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: Freestanding micro tubes (1.5 mL) containing 0.7 mL of toxin.

10. Storage Conditions: Store at -50° to -80°C.

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

12. Origin and Passage History: *C. novyi* type B strain CN 234.3 (IRP 79), used to produce IRP 636, was obtained from Wellcome Research Laboratories, Beckenham, England, on July 16, 1965. The number of passages is unknown.

13. Method of Preparation: Culture CN 234.3 (IRP 79) 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 proteose peptone, trypticase, yeast extract, and liver powder. Actively growing culture was aseptically added to the inside of the dialysis membranes and incubated at 35°C for 120-140 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 45-60 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: VS.DB.CVB.Reagent.Requests@aphis.usda.gov

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