

United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
P. O. Box 844
Ames, IA 50010

ATTENTION:
Select Agent shipping
requirements must be
met for >10 vials

1. **Reagent Name:** *Clostridium botulinum* Type C Toxin
2. **Strain or Source:** Not applicable
3. **Lot Number:** IRP 583
4. **Fill Date:** January 24, 2012
5. **Expiration Date:** No expiration date has been assigned to this product because *C. botulinum* type C toxin has demonstrated over time to be very stable if properly stored.

Precautions: Exposure to the toxin of *C. botulinum* is a primary hazard. The toxin may be absorbed after ingestion or following contact with the skin, eyes, or mucous membranes, including the respiratory tract.

6. **Intended Use:** To serve as the standard toxin for potency testing of *C. botulinum* type C bacterin-toxoids.
7. **Instructions for Use:** *C. botulinum* type C toxin IRP 583 diluted 1:10 is considered the standard toxin dilution when conducting potency tests in mink as outlined in title 9, *Code of Federal Regulations* (9 CFR), part 113.110. Prepare the dilution by adding 1.0 mL of IRP 583 to 9.0 mL of sterile Phosphate buffer saline (PBS) with 0.2% gelatin, pH 7.2. IRP 583 diluted 1:10 contains $10^{4.0}$ mouse minimum lethal dose (MLD) per 0.5 mL.

8. Test of Reagent:

Determination of MLD - White female mice weighing 16-23 grams were injected in the intraperitoneal cavity with 0.5 mL of toxin diluted in PBS with 0.2% gelatin. The toxin was found to contain $10^{4.0}$ mouse minimum lethal dose (MLD) per 0.5 mL when diluted 1:10.

LD₅₀ Determination - White female mice weighing 16-23 grams were injected in the intraperitoneal cavity with 0.5 mL of toxin diluted in PBS with 0.2% gelatin. The toxin was found to contain 1:230,000 LD₅₀ per 0.5 mL dose.

Sterility test - The toxin was tested for sterility and found to be free of viable bacteria and fungi.

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

10. Storage Conditions: Store IRP 583 at $-70^{\circ}\pm 5^{\circ}\text{C}$.

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

12. Origin and Passage History: *C. botulinum* type C (African strain) 203A was used to produce IRP 583. It was obtained in June 1968 from Fromm Laboratories, 703 Lakeshore Road, Grafton, WI 53024.

13. Method of Preparation: Dialysis membranes with a molecular weight cutoff of 12,000-14,000 daltons were filled with PBS and suspended in 1-liter trypsinizing flasks containing proteose peptone-trypticase-yeast extract media with dextrose. The flasks were autoclaved at 121°C for 25 minutes, cooled, and incubated at 35°C in an atmosphere containing 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. Approximately 0.5 mL of actively growing culture 203A was aseptically added to the inside of each dialysis membrane and incubated for 120 hours in an anaerobic atmosphere as previously described. The culture was centrifuged at $10,000 \times g$ for 60 minutes. The culture supernatant was adjusted to pH 6.8 and passed through a Millipore filtration unit containing a 0.22- μm membrane. The toxin was diluted 1:2.5 with 1X Phosphate buffered saline and glycerol was added to a final concentration of 15% volume/volume.

14. Other: Request must be accompanied with a completed APHIS/CDC Form 2 if requesting over 10 vials.

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

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

REVISED: 29Mar18 tlt