**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 >1 vial**

**1. Reagent Name:** *Clostridium botulinum* Type B Toxin

**2. Strain or Source:** Not applicable

**3. Lot Number:** IRP 430

**4. Fill Date:** June 6, 1995

**5. Expiration Date:** No expiration date has been assigned to this product because *C. botulinum* type B 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:** IRP 430 serves as the standard toxin when conducting *C. botulinum* type B toxin-neutralization (TN) tests in mice.

**7. Instructions for Use:** To conduct TN tests in mice at the 0.005 antitoxin unit per mL (AU/mL) level dilute IRP 430 1:120,000. Prepare the dilution by transferring 1.0 mL of IRP 430 to 9.0 mL of diluent, transferring 1.0 mL of the 1:10 dilution to 9.0 mL of diluent, transferring 1.0 mL of the 1:100 dilution to 9.0 mL of diluent, transferring 1.0 mL of the 1:1,000 dilution to 9.0 mL of diluent, and transferring 1.0 mL of the 1:10,000 dilution to 11 mL of diluent. Prepare twofold dilutions of standard antitoxin (0.01, 0.005, 0.0025 AU/mL) and add 1.5 mL of antitoxin to 1.5 mL of toxin. Prepare 1:4 dilutions of the test serum (e.g., undiluted, 1:4, 1:16, 1:64) and add 1.5 mL of each dilution to 1.5 mL of toxin. Inject mice intraperitoneally (i/p) with 0.5 mL of the toxin-antitoxin mixture. Record the deaths of injected mice during a 4 day period. Calculate the 50% end points of the standard antitoxin and test serum by the Reed and Muench method. The units per mL in the test serum are equal to its 50% endpoint dilution multiplied by the units per mL of the standard antitoxin at its calculated 50% endpoint.

To conduct toxin TN in mice using $L_o$ and $L_+$ doses of toxin at the 1.0 AU/mL level dilute IRP 430 1:20. Prepare the 1:20 dilution by transferring 1.0 mL of well mixed IRP 430 to 19 mL of diluent. Prepare the 1.0 $L_o$ dose by adding 2.0 mL of standard antitoxin containing 1.0 AU/mL to 1.0 mL of standard toxin diluted 1:20 and adjusting the total volume to 4.0 mL with 1.0 mL of diluent. Prepare the 1.0 $L_+$ dose by adding 2.0 mL of standard antitoxin containing 1.0 AU/mL to 1.6 mL of standard toxin diluted 1:20 and adjusting the total volume to 4.0 mL with 0.4 mL of diluent. Inject mice (i/p) with 0.5 mL of the toxin-antitoxin mixture and record the deaths for 4 days. Prepare all dilutions in sterile 0.067 M phosphate buffered saline (PBS) with 0.2% gelatin, pH 7.2.

**8. Test of Reagent:**

*Determination of $LD_{50}$* - White female mice weighing 16-20 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 $10^{6.462}$ mouse intraperitoneal lethal dose fifty ($LD_{50}$) per 0.5 mL.

*Determination of test dose of toxin* - The test dose of toxin used in the 50% endpoint calculation was determined by preparing dilutions of IRP 430 over a range within which the endpoint was anticipated. One and one-half mL of *C. botulinum* type B antitoxin, containing 0.005 International Unit (IU) per mL, was added to each tube containing 1.5 mL of diluted toxin. Mice were injected in the intraperitoneal cavity with 0.5 mL of the toxin-antitoxin mixture and deaths recorded for 4 days. The 50% endpoint dilution of the test toxin was calculated by the method of Reed and Muench.

The $L_o$ and $L_+$ doses were established by injecting mice in the intraperitoneal cavity with 0.5 mL of a mixture containing varying amounts of IRP 430 combined with 1.0 IU of *C. botulinum* type B antitoxin. The $L_o$ dose for the TN test is the largest amount of toxin which can be mixed with 1.0 IU of antitoxin and not cause death in injected mice within 96 hours. The $L_+$ dose is the smallest amount of toxin which can be mixed with 1.0 IU of antitoxin and cause death in at least 80% of injected mice within 96 hours.

*Sterility test* - The toxin was tested for sterility and found to be free of viable bacteria and fungi according to procedures outlined in title 9, *Code of Federal Regulations* (9 CFR), section 113.26.

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

**10. Storage Conditions:** Store IRP 430 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. botulinum* type B strain 79E-230 (BBL No. 102) was used to produce IRP 430. The culture was obtained from the University of Kentucky, Lexington, Kentucky, in April 1994.

**13. Method of Preparation:** *C. botulinum* type B strain 79E-230 was cultivated in proteose peptone-trypticase-yeast extract media with dextrose for 120 hours at 35ºC in an anaerobic atmosphere containing 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. The 120-hour 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 culture filtrate was treated with ammonium sulfate ($[NH_4]_2SO_4$), and the precipitate was suspended in deionized water and dialyzed against 0.015 M PBS, pH 6.8. The dialyzed material was passed through a Millipore filtration unit containing a 0.22-µm membrane and glycerol added to a final concentration of 10% (v/v).

**14. Other: <u>Request must be accompanied by a completed APHIS/CDC Form 2.</u>**

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