

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 perfringens* type A recombinant-alpha antitoxin
2. **Strain or Source:** Not applicable
3. **Lot Number:** IRP 564 (08)
4. **Fill Date:** September 17, 2008
5. **Expiration Date:** February 28, 2029

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

6. **Intended Use:** IRP 564 (08) serves as the standard antitoxin when *C. perfringens* type A (alpha) toxin-neutralization (TN) tests in mice.
  7. **Instructions for Use:** IRP 564 (08) contains 55 International units of alpha antitoxin per mL. A dilution of standard antitoxin containing 1.0 antitoxin unit per mL (AU/mL) is used in the mouse assay. The dilution is prepared by adding 1.0 mL of IRP 564 (08) to 9.0 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The antitoxin diluted 1:10 should be dispensed into individual tubes and stored at  $-70^{\circ}\pm 5^{\circ}\text{C}$ . The final dilution containing 1.0 AU/mL for the mouse assay is prepared by adding 1.0 mL of the 1:10 dilution to 4.5 mL of diluent.
  8. **Test of Reagent:** *Determination of antitoxin titer* - The antitoxin titer of IRP 564 (08) was determined by injecting mice intravenously with 0.5-mL volumes of diluted antitoxin mixed with 1.0 L<sub>+</sub> dose of toxin (the smallest amount of toxin which, when mixed with 1.0 unit of antitoxin causes death in at least 80% of the mice within 48 hours) and 1.0 L<sub>o</sub> dose of toxin (the largest amount of toxin which, when mixed with 1.0 unit of antitoxin, causes no deaths in animals within 48 hours). The antitoxin titer of IRP 564 (08) was confirmed by comparing the results of mice injected with toxin-antitoxin mixtures containing 1.0 mL of IRP 564 (08) possessing 1.0 AU/mL with the results of mice injected with toxin-antitoxin mixtures containing 1.0 mL of International *C. perfringens* alpha antitoxin possessing 1.0 AU/mL.
- 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:** 2-mL glass vials containing 1.3 mL of antitoxin.

- 10. Storage Conditions:** Store at  $-70^{\circ}\pm 5^{\circ}\text{C}$ .
- 11. CVB Technical Contact:** Bacteriology Section, Center for Veterinary Biologics, (515) 337-6100.
- 12. Origin and Passage History:** *C. perfringens* type A recombinant-alpha antitoxin IRP 564-08 was obtained from young adult goats immunized with IRP 566 (*C. perfringens* type A recombinant-alpha toxoid) and antigen prepared from IRP 563-07 (*C. perfringens* type A recombinant-alpha toxin).
- 13. Method of Preparation:** Goats with no history of Clostridial vaccinations received multiple injections of *C. perfringens* type A recombinant-alpha toxoid (IRP 566) and toxin (IRP 563) during a 6-month period. Sera from the hyperimmunized goats were fractionated with ammonium sulfate. The immunoglobulin obtained was dialyzed against 0.010 M phosphate buffered saline, pH 7.2, passed through a sterile disposable filter unit containing a 0.22- $\mu\text{m}$  membrane, aseptically dispensed into sterile vials, and stored at  $-70^{\circ}\pm 5^{\circ}\text{C}$ . No preservatives were added to the product.
- 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](mailto:VS.DB.CVB.Reagent.Requests@usda.gov)

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