

**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 D (epsilon) toxin
2. **Strain or Source:** Not applicable
3. **Lot Number:** IRP 450
4. **Fill Date:** September 13, 1999
5. **Expiration Date:** No expiration date has been assigned to this product because *C. perfringens* type D (epsilon) toxin has demonstrated over time to be very stable if properly stored. The stability of this reagent will be routinely monitored by the Center for Veterinary Biologics.

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 450 serves as the standard toxin when conducting *C. perfringens* type D (epsilon) toxin-neutralization tests in mice.

7. **Instructions for Use:**

Mouse assay: IRP 450 diluted 1:320 is considered the standard toxin dilution when conducting toxin-neutralization tests in mice as outlined in 9 CFR 113.112 and 9 CFR 113.455. The dilution is prepared by adding 1.0 mL of IRP 450 to 31 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The toxin is further diluted to 1:320 by adding 1.0 mL of the 1:32 dilution to 9.0 mL of diluent. A volume of 0.6 mL of the toxin diluted 1:320 and 0.4 mL of diluent is equivalent to 10 L_o doses. A volume of 0.9 mL of toxin and 0.1 mL of diluent is equivalent to 10 L₊ doses. *C. perfringens* type D (epsilon) toxin IRP 450 diluted 1:32 is stable when stored at -70°± 5°C.

MDCK cell assay (Final Product): IRP 450 diluted 1:320 is considered the standard toxin dilution when conducting the MDCK cell assay as outlined in BBPRO1008. The dilution is prepared by adding 1.0 mL of IRP 450 to 31.0 mL of Minimal Essential Medium (MEM) with Earles F-15 with 0.5% LAH, containing 1% L-glutamine and 0.1% pen-strep and 5% fetal bovine serum. The toxin is further diluted to 1:320 by adding 1.0 mL of the 1:32 dilution to 9.0 mL of diluent; this dilution is considered the standard toxin preparation and is used in the toxin

control wells. *C. perfringens* type D (epsilon) toxin IRP 450 diluted 1:32 is stable when stored at $-70^{\circ} \pm 5^{\circ}\text{C}$. Dilute the standard toxin preparation further according to the following table:

Tube #	microliters of 1:320 toxin	DMEM in mL	final dilution
1	239	2	1:3000
2	267	2	1:2700
3	308	2	1:2400
4	360	2	1:2100
5	432	2	1:1800

Add the graduated toxin to each column according to the following template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Toxin diluted 1:3000	Toxin diluted 1:2700	Toxin diluted 1:2400	Toxin diluted 1:2100	Toxin diluted 1:1800	Live cell Control	Toxin diluted to 1:2700	Toxin diluted to 1:2400	Toxin diluted to 1:2100	Toxin diluted to 1:1800	
C												
D												
E												
F												
G												
H												

8. Test of Reagent:

Determination of test dose of toxin - The L_0 and L_+ doses were established by injecting mice intravenously with 0.2 mL of a mixture of varying amounts of IRP 450 combined with 0.1 unit of standard epsilon antitoxin. The L_0 dose for the *C. perfringens* type D (epsilon) 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 24 hours. The L_+ dose 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 24 hours.

Determination of LD_{50} - Harlan Sprague Dowley female mice weighing 16-20 g were injected intravenously with 0.2 mL of toxin diluted in peptone diluent. The toxin was found to contain $10^{5.054}$ mouse lethal dose fifty (LD_{50}) 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 450 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 D antitoxin.

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: Three-mL glass vials each containing 1.3 mL of toxin.

10. Storage Conditions: Store 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. perfringens* type D (epsilon) culture CN3688, used to produce IRP 450, was obtained from Coopers Animal Health, Inc., 1201 Douglas Avenue, Kansas City, KS 66103-1438, on January 5, 1976. The number of passages is unknown.

13. Method of Preparation: Culture CN3688 was grown in a 40-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 6 hours. The culture was centrifuged and the supernatant passed through a Millipore filtration unit containing a $0.22\text{-}\mu\text{m}$ membrane. The filtrate was further processed using a Millipore pellicon cassette system containing a high volume ultrafilter. The concentrated toxin was adjusted to pH 6.8 and passed through a sterile Millipore filtration unit containing a $0.22\text{-}\mu\text{m}$ membrane. Sterile glycerol was added to the product at a final concentration of 5% v/v.

14. Other:

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

Reagent orders forms (APHIS 2018) are available from:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

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