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 632

4. Fill Date: June 2, 2015

5. Expiration Date: February 28, 2029

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

7. Instructions for Use:

Mouse Assay – IRP 632 diluted 1:150 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 0.5 mL of IRP 632 to 4.5 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The toxin is further diluted to 1:150 by adding 1.0 mL of the 1:10 dilution to 14.0 mL of diluent. A volume of 0.6 mL of the toxin diluted 1:150 and 0.4 mL of diluent is equivalent to 10 Lo doses. A volume of 0.9 mL toxin diluted 1:150 and 0.1 mL of diluent is equivalent to 10 L+ doses. *C. perfringens* type D (epsilon) toxin IRP 632 diluted 1:10 is stable when stored at -60°C or lower.

MDCK cell assay (Final Product) – IRP 632 diluted 1:150 is considered the standard toxin dilution when conducting the MDCK cell assay as outlined in **CVB-PRO-0008, Potency Test for Clostridium perfringens Type D Epsilon Antitoxin Using a Cell Assay**. The dilution is prepared by adding 1.0 mL of IRP 632 to 9.0 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 1:150 by adding 1.0 mL of the 1:10 dilution to 14.0 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 632 diluted 1:10 is stable when stored at -60°C or lower. Dilute the standard toxin preparation further according to the following table:

Tube #	microliters of 1:150 toxin	DMEM in mL	final dilution
1	154	2	1:2100
2	182	2	1:1800
3	222	2	1:1500
4	286	2	1:1200
5	400	2	1:900

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		**Standard Antitoxin**						**Unknown Antitoxin**				
B		Standard Toxin diluted 1:2100	Standard Toxin diluted 1:1800	Standard Toxin diluted 1:1500	Standard Toxin diluted 1:1200	Standard Toxin diluted 1:900	Live cell Control	Standard Toxin diluted 1:1800	Standard Toxin diluted 1:1500	Standard Toxin diluted 1:1200	Standard Toxin diluted 1:900	
C												
D												
E							Toxin Control					
F												
G												
H												

8. Test of Reagent:

Determination of test dose of toxin – The Lo and L+ doses were established by injecting mice intravenously with 0.2 mL of a mixture of varying amounts of IRP 632 combined with 0.1 unit of standard epsilon antitoxin. The Lo 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 LD50 – Swiss Webster 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 $10^{4.717}$ mouse lethal dose fifty (LD50) 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 632 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: 0.8 mL aliquots of toxin in plastic microtubes.

10. Storage Conditions: Store at -60°C or lower.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6100.

12. Origin and Passage History: *C. perfringens* type D (epsilon) culture CN3688, used to produce IRP 632, 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 14-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-µm 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-µm membrane. Sterile glycerol was added to the product at a final concentration of 5% v/v.

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

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