



Data sheet *Clostridium perfringens* Type C (beta) toxin

Benten Biotech SRL
Parque Tecnológico- LATU.
Montevideo, Uruguay
CP 11500

1. Reagent name: *Clostridium perfringens* C (beta) toxin

2. Strain or resource: Not apply

3. Lot number: TCPC 003/21

4. Fill date: July. 2021

5. Expiration date: The expiration date was not evaluated. According to the literature, the toxin is very stable and could be active for several years.

Precautions: This reagent does not represent a risk for laboratory personnel working with the toxin if fundamental laboratory techniques are followed.

6. Intended to use: Used as a standard antitoxin when evaluating *C. perfringens* C toxin in the neutralization test in mice.

7. Instruction for use:

Mouse assay: TCPC 003/21 diluted 1:47 is considered the standard toxin dilution when conducting toxin-neutralization tests in mice. The dilution is prepared by adding 2 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2) to the TCPC 003/21 freeze dry toxin vial. Make the first dilution 1/10, dilute 0,5 mL of well mixed TCPC 003/21 in 4,5 mL of peptone diluent. The L+ dose is prepared by adding 1 ml of 1/10 TCPC 003/21 toxin dilution and 3,7 ml of peptone diluent. The Lo dose is prepared by adding 1 ml of 1/10 TCPC 003/21 toxin dilution and 4,2 ml of peptone diluent. *C. perfringens* type C (beta) TCPC 003/21 toxin diluted 1:10 is stable when stored at -70°.

8. Reagent test:

Determination of antitoxin titer- Antitoxin titer was determined by injecting 16-20 g mice intravenously with 0.2 ml volume of diluted antitoxin mixed with 1 dose of L + toxin (the least amount of toxin when mixed with 1 unit of antitoxin, causes death in at least 80% of animals in 72 hours) and 1 dose of Lo of toxin (the highest amount of toxin that, when mixed with 1 unit of antitoxin, does not cause animal death within 72 hours).



Determination of LD50 - Female mice weighing 16-20 g were injected intraperitoneally with 0.5 mL of toxin diluted in peptone diluent.

Determination of toxin type - The toxin type was confirmed by performing toxin-neutralization tests in mice. The mice were injected intravenously with mixtures of TCPC 003/21 and *C. perfringens* type A, C, or D antitoxin. All of the mice died within 24 hours except those receiving mixtures containing type C antitoxin.

Sterility test: For the *Clostridium perfringens* C toxin, the absence of viable bacteria and fungi was analyzed according to the procedures described in 9 CFR (USA).

9. Container size, type, weight or volume: 10 mL vial containing 2 ml of lyophilized toxin.

10. Storage conditions: store at -80 °C.

11. Technical contact Benten Biotech: Quality Control Section, +59899178546.

12. Origin and history of passage: *C. perfringens* type C (beta) culture ATCC 3628, used to produce TCPC 003/21, was obtained from ATCC. The number of passages is unknown.

13. Preparation method: Culture ATCC 3628 was grown in a 2.5 -litre Sartorius Biostat A fermentor containing media consisting of triptone, proteose peptone, and yeast extract. Actively growing culture was aseptically added to the fermentor and incubated at 35°C for approximately 4 hours. The culture was centrifuged 4,000 x g for 60 minutes and the supernatant was passed through 1.2, 0.8, 0.4 and 0.2 µm Sartorius sterile filters. The filtrate was further concentrated then times by 10 KDa ultrafiltration Millipore pellicon cassette. The concentrated toxin was adjusted to pH 6.8 and passed through a 0.2 µm Sartorius filter. Sterile skim milk was added to the product at a final concentration of 50% v/v and 1:10000 thimerosal was added and toxin was fractionated in 2 mL each vial. The vials were freeze-dried with Christ equipment.

14. Other: Transport according to UN3172 (Biological Toxins).

Reagent orders and feedback to the following email address: info@bentenbiotech.com

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