

Data sheet *Clostridium novyi* type B (alpha) toxin

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

1. Reagent name: *Clostridium novyi* type B (alpha) toxin

2. Strain or resource: Not apply

3. Lot number: TCN 002/20B

4. Fill date: Dec 2020

5. Expiration date: No expiration date was not evaluated. According to the literature, the toxin is 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: Serves as standard toxin when conducting *C. novyi* toxin neutralization tests in mice.

7. Instruction for use:

Mouse assay: TCNB 002/20B diluted 1:8 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 TCNB 002/20B freeze dry toxin vial. The L+ dose is prepared by adding 1 mL of well mixed TCNB 002/20B in 7 mL of peptone diluent. The Lo dose is prepared by adding 1 ml of TCNB 002/20B toxin in 9 ml of peptone diluent.

8. Reagent test:

Determination of toxin titer- Doses Lo and L+ were established by injecting mice intravenously with 0.2 ml of a mixture of varying amounts of TCNB 002/20B combined with 1 international unit (IU) of *C. novyi* B antitoxin. Dose 1 Lo for the *C. novyi* B antitoxin toxin neutralization test is the highest amount of toxin that can be mixed with 1 IU antitoxin and not cause death in injected mice within 72 hours. The 1 L + dose for the *C. novyi* B antitoxin-toxin neutralization test is the minimum amount of toxin that can be mixed with 1 IU of *C. novyi* B antitoxin and cause death in at least 80% of mice injected into the 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 TCNB 002/20B and *C. perfringens* type A, C, or D antitoxin. All of the mice died within 24 hours except those receiving mixtures containing *C. novyi* B antitoxin.

Sterility test: For the *Clostridium novyi* 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. novyi* type B culture was used to produce TCNB 002/20B toxin. The number of times the culture has been passed is unknown.

13. Preparation method: Culture ATCC 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

REVISED: July 2021