Instruction Manual

ELISA for the detection of Clostridium difficile Toxin A AND Toxin B in suspensions

Product Code: TGC-E001-1

- For Researche Use Only -

I. Kit reagents supplied by tgcBIOMICS:

1.	**ELISA plate** coated with anti-ToxinA and anti-ToxinB antibodies		store at 2 - 8°C
2.	**Dilution Buffer**	50 ml ready to use	store at 2 - 8°C
3.	**Positive Control** : ToxinA&B	2 ml ready to use	store at 2 - 8°C
4.	**Conjugate** : anti ToxinA&B-HRP	7 ml ready to use	store at 2 - 8°C
5.	**10x Wash Buffer**	30 ml to be 10x diluted	store at 2 - 8°C
6.	**TMB - Substrate**	14 ml ready to use	store at 2 - 8°C
7.	**Stop reagent**	7,5 ml ready to use	store at 2 - 8°C

II. To be prepared in advance of testing:

1. Prepare 1x Wash Buffer: the Wash Buffer is supplied as a 10x concentrate. The 30 ml supplied need to be diluted to a total volume of 300 ml by adding 270 ml distilled water. Preparation of aliquots of the Wash Buffer is done accordingly. Store your diluted 1x Wash Buffer between 2°C and 8°C to avoid growth of contaminating microbes.

2. Microtitre plate: the plates are sealed in aluminum bags that need to be resealed once opened. Before starting determine the number of wells to be used. Do not contaminate the wells with your fingers. The plates can be used as broken “single wells” or in form of single strips. Each strip contains 8 wells coated with antibodies specific for C. difficile ToxinA and ToxinB. Assay wells not used should immediately be returned to the bag and carefully resealed with desiccant. After opening stability of plates at 4°C will be about 6 months.

III. Preparing the samples

Culture supernatant:
Centrifuge the Clostridium difficile culture at 2500 G for 2-5 minutes and dilute the supernatant 1:2 to 1:10 in Dilution Buffer.

Colonies:
For testing colonies from freshly grown agar plates remove app. 5 colonies or 1 cm² of a confluent plate and suspend the bacteria in 1ml Dilution Buffer. Homogenize the suspension by vortexing and centrifuge the sample at 2500 G for 2-5 minutes. The supernatant can be used directly in the test, without further dilution.

Stool sample:
Transfer about 50 µl liquid stool sample or take an equivalent amount (50 mg) of compact stool in 450 µl Dilution Buffer, homogenize the suspension by vortexing and centrifuge the sample at 2500 G for 2-5 minutes. The supernatant can be used directly in the test, no further dilution is needed.

IV. Test Procedure

Wear gloves for all manipulations with potentially contaminated or toxic suspensions.

All reagents must be at room temperature prior to their use in the assay:

1. Pipette 100 µl of the prepared specimen or the control toxin into each single well.
   A diagram showing the dilutions routinely used for ToxinA und ToxinB calibration is attached.
   As negative control use 100 µl of the Dilution Buffer.

2. Add 50 µl of the conjugate anti Toxin A&B-HRP to each well to detect Toxin A and Toxin B

   After the addition of the conjugate pipette once up and down to mix the components

3. Incubate specimen plus conjugate for 60 min at 37°C.

4. Wash each well 3 x with Wash Buffer. After each washing, completely remove any residual liquid by striking the plate (wells) onto a dry paper.

5. Thereafter add 100µl substrate to each well

6. Incubate for 20 min at RT

7. The color development will be stopped by adding 50 µl STOP-solution to each well.

8. Reading the Optical Density will be with a spectrophotometer at 450 nm and 620 nm.

V. Interpretation of results:

Measurement is at 450nm and 620nm:

- The read out of the assay is based on the measurements of the optical density at 450 nm and 620 nm and is calculated as OD_{450}-OD_{620}.

Negative control:

- The $OD_{450-620}$ background should be below 0,100

Positive control:

- The $OD_{450-620}$ of the positive control should be >1,00.

Cut off:

- The cut off with a negative control of < 0,05 is: **0,2 $OD_{450-620}$**

Contact address to order and for further requests

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