tgcBIOMICS
Targeted Genetic Conditioning

Instruction Manual

ELISA for the separate detection of *Clostridium difficile* Toxin A OR Toxin B in suspensions

C. diff. Toxins A or B quanti

Product Code: TGC-E002-1

- For Researche Use Only -

I. Kit reagents supplied by tgcBIOMICS:

1.	**ELISA plate** coated with anti-toxin A and anti-toxin B antibodies		store at 2 – 8 °C
2.	**Dilution Buffer**	50 ml ready to use	store at 2 – 8 °C
3.	**Standard control A (80 ng/ml)**	1,0 ml ready to use	store at 2 – 8 °C
	Standard control B (80 ng/ml)	1,0 ml ready to use	store at 2 – 8 °C
4.	**Conjugate 1**: anti-Toxin A-HRP	3,5 ml ready to use	store at 2 – 8 °C
	Conjugate 2: anti-Toxin B-HRP	3,5 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. diff.* toxin A and oxin B. 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.

3. Choose your conjugate properly:

    - for the detection of toxin A use the anti-toxin A-HRP conjugate
    - for the detection of toxin B use the anti-toxin B-HRP conjugate

    as supplied in separate solutions.

    The conjugate is ready to use. For each well you need 50 µl.

III. Preparing the samples

Culture supernatant:
Centrifuge the *C. diff.* 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 0,5 ml 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.

Standard control:
The range of the measurement of toxin A and toxin B is different.
For an efficient dilution of the standard control toxins see the attached diagram.

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 (for a calibration the controls have to be prediluted) into each single well.
   A diagram showing the dilutions routinely used for Toxin A und Toxin B calibration is attached.
   As negative control use 100 µl of the Dilution Buffer.

2. Add 50 µl of the conjugate anti-Toxin A-HRP to each well to detect Toxin A.
   OR
   Add 50 µl of the conjugate anti-Toxin B-HRP to each well to detect 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 100 µl 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 done with a spectrophotometer at 450 nm and 620 nm.

V. Interpretation of results:

Measurement of the optical density is at 450 nm and 620 nm:

- Background at 620 nm is subtracted from the 450 nm value.

Negative control:

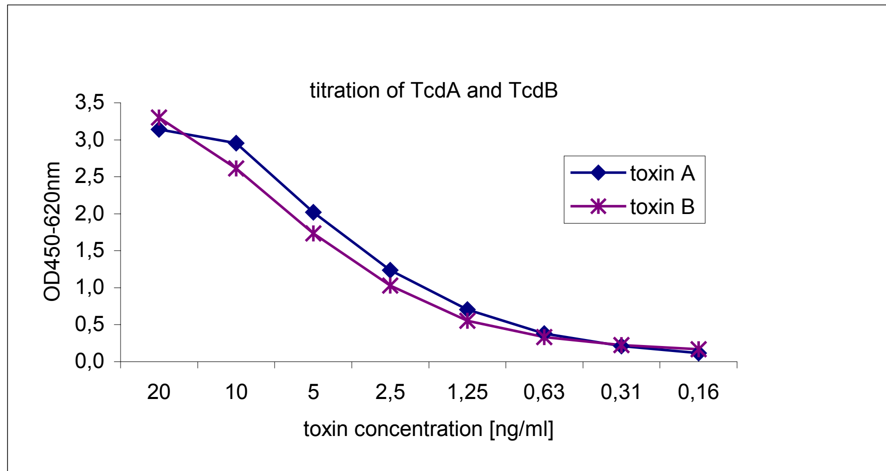
- The $OD_{450-620}$ of the negative control normally is at 0,020 to 0,050; should be below $OD_{450-620}$ 0,100.

Standard control:

The standard control toxins are recombinant toxins. The concentration of 80 ng/ml is an equivalent to the native *C. diff.* toxins by tgcBIOMICS determind by Bradford.

- The standard toxins can be used as a positiv control (ready to use).
  OR
- The standard toxins can be used to generate a calibration curve. Please see the attached diagram for an example of typical standard toxin A and B titration.

VI Calibration with standard toxins



Standard toxin titrating curve

The results of the *C. diff.* toxins A and B will depend upon lifetime of the assay, processing and incubation (time; temperature; etc.), so insignificant shiftings are possible .To generate a correct toxin calibration its ingenious to test probes and calibration in one measurement.

Sensitivity of the test is (test reaction > 3-fold background reaction):

TcdA	-	0,31 ng/ml
TcdB	-	0,31 ng/ml

Cut off with a negative control of < 0,05 is:

~0,2 $OD_{450\text{-}600}$

Contact address to order and for further requests

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