tgcBIOMICS
Targeted Genetic Conditioning

Instruction Manual

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

C. difficile toxins A or B quanti

Product Code: TGC-E002-1

- For Researche Use Only -

I. Kit reagents supplied by tgcBIOMICS

All components should be stored at 4 °C

1. **ELISA plate** coated with anti-toxin A and anti-toxin B antibodies
2. **Dilution Buffer** 50 ml ready to use
3. **Standard control A (80 ng/ml)** 1,0 ml ready to use
4. **Standard control B (80 ng/ml)** 1,0 ml ready to use
5. **Conjugate 1**: anti-toxin A-HRP 3,5 ml ready to use
6. **Conjugate 2**: anti-toxin B-HRP 3,5 ml ready to use
7. **10x Wash Buffer** 50 ml to be diluted
8. **TMB - Substrate** 14 ml ready to use
9. **Stop reagent** 7,5 ml ready to use

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II. To be prepared in advance of testing

1. Preparation of 1x Wash Buffer:
The Wash Buffer is supplied as a 10x concentrate. Determine the amount needed and dilute the concentrate 1+9 with distilled water. It is possible to prepare the Wash Buffer in greater amounts that could be stored for later use for several weeks at 4 °C.

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 touch 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* toxin A and toxin B. Wells not used should immediately be returned to the bag and carefully resealed with desiccant. Once opened stability of plates at 4 °C will be about 6 months.

3. Selection of the Conjugate:
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.

Both are supplied in separate solutions and ready tu use. For each well 50 µl will be needed.

III. Preparing the samples

Important notes before starting:
If the ELISA procedure should follow the one step protocol, it is essential that the test sample is totally free of PBS. To avoid high background reactions resulting from mixing of PBS and conjugate, the two step protocol is strongly recommended. In this case, the anti toxin A or B-HRP conjugate has to be prediluted with two parts dilution buffer (for example, 100 µl of the anti toxin A or B-HRP conjugate with 200 µl dilution buffer).

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

2. Colonies:
For testing colonies from freshly grown agar plates remove app. 5 colonies or 1 cm² of a confluent plate and resuspend 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 without further dilution.

3. 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 suction and ejection from a disposable pipette or by vortexing. After leaving for a short time to allow sedimentation of stool particles the clarified supernatant can be used directly in the test. Automated equipment may be used with specimens that have been centrifuged 5 min by 2500 x g to remove any particulate matter.

4. Standard control:
An example for routinely used dilutions of the standard control toxins is given in the diagram below.

IV. Test Procedure

Warnings and precautions:
All reagents and materials which come into contact with potentially infectious samples must be treated with suitable disinfectant or autoclaved; suitable disposable gloves must be worn during the entire test.

All reagents should be at room temperature prior to use:

A: One step protocol

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 and toxin B calibration is attached.
   As negative control use 100 µl of the Dilution Buffer.
2. Add 50 µl of the anti-toxin A-HRP conjugate to detect toxin A
   OR 50 µl of the anti-toxin B-HRP conjugate to detect toxin B to each well.
3. Incubate specimen plus conjugate for 60 min at 37 °C.
   Continue with step 4 of the protocol.

B: Two step protocol

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 and toxin B calibration is attached.
   As negative control use 100 µl of the Dilution Buffer.
2'. Incubate for 60 min at 37 °C.
3'. 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.
4'. Add 100 µl of the anti toxin A **OR** B-HRP conjugate (prediluted with two parts dilution buffer; see section III) to each well to detect toxin A or toxin B.
5'. Incubate for 30 min at 37 °C.
   Continue with step 4 of the protocol.

4. Wash each well 3 x with Wash Buffer. After each washing step, completely remove any residual liquid by striking the plate (wells) onto a dry paper.
5. Thereafter add 100 µl of substrate to each well.
6. Incubate for 15 min at RT.
7. The color development will be stopped by adding 50 µl Stop solution to each well.
8. Measurement of the extinction will be done with a microtiter plate photometer at 450 nm versus 620 nm.

V. Interpretation of results

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

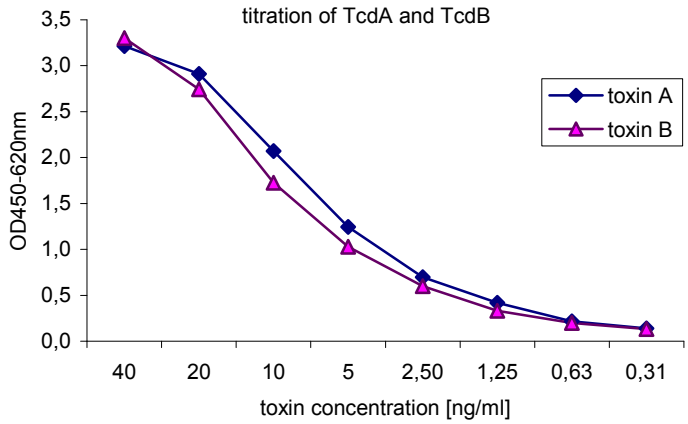
- Negative control:
  The $OD_{450-620}$ of the negative control should be below 0,100.

- Standard controls:
  The standard control toxins are recombinant toxins with a concentration of 80 ng/ml (equivalent to the native *C. difficile* toxins) as determined by the method of Bradford.

- The standard toxins can be used as a ready to use positive control
  OR
- to generate a calibration curve as exemplified in the diagram below.

- Cut off value:
  The cut off of the assay is the OD of the negative control + OD 0,100
  For example, with a negative control value of $OD_{450-620}$ 0,030 the cut off is 0,130.

VI. Calibration with standard toxins



Typical titration curve generated with the recombinant standard toxins

Note: the results for the *C. difficile* toxins A and B will depend upon several factors (e. g. incubation time and temperature, etc.), so minor deviations are possible. For proper toxin calibration it is recommended to assay samples and standards in one reading.

Sensitivity of the test is for: TcdA 0,5 ng/ml
TcdB 0,5 ng/ml