tgcBIOMICS
Targeted Genetic Conditioning

Instruction Manual

ELISA for the simultaneous detection of *Clostridium difficile* toxin A and B in stool

-For *In Vitro* Diagnostic Use -

Product Code: TGC-E001-1

Intended use:

The test is an *in vitro* diagnostic enzyme immunoassay for the detection of toxin A and toxin B produced by toxigenic strains of *Clostridium difficile* in human feces.

Introduction:

C. difficile is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered due to treatment with antibiotics. Subsequently, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis.

The clinical symptoms associated with the disease are believed to be primarily due to the tissue-damaging enterotoxin A (TcdA), whereas the cytotoxin (TcdB) is the one detected by the cell culture cytotoxicity assay. Most strains produce both toxins, although clinically relevant toxin A negative/toxin B positive strains have been isolated with increasing frequency worldwide.
Laboratory diagnosis of *C. difficile* infection is most commonly performed in a two-step algorithm: (1) screening of *C. difficile* presence using an immunoassay for the detection of *C. difficile* glutamate dehydrogenase (GDH) followed by (2) assaying the presence of toxins A/B using either an immunoassay and/or by PCR based techniques, the latter especially important in cases where the GDH test is positive but the toxin ELISA results negative. This could be the case for toxin production below the detection limit or capture of toxins by anti-toxin antibodies.

The tgcBIOMICS ELISA kit *C. difficile* A and B is an ELISA-based test for detection of *C. difficile* toxins A and B in fecal specimens.

Contact address (technical support/order information):

tgcBIOMICS GmbH
Franz-Kirsten-Str. 1, Gebäude 2
D-55411 Bingen
Tel: +49-6721-98419-0
FAX: +49-6721-98419-18
e-mail: info@tgcbiomics.de

Principle of the test:

The test uses a mixture of two different monoclonal antibodies, each highly specific for one of the two *C. difficile* toxins and immobilized on the microassay plate. For detection, a second pair of monoclonal antibodies conjugated to horseradish peroxidase is used. In the assay, an aliquot of a fecal specimen is emulsified in the diluent and transferred to the microwell together with the conjugate mix. If antigen is present in the specimen, it will bind to the immobilized mAbs and the conjugate mix during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of substrate, a color is detected due to the enzyme-antibody-antigen complexes that form in the presence of toxin A and/or B.

Materials provided:

1. **Microassay plate:** coated with a toxin A/B antibody mix
2. **Dilution buffer:** 50 ml ready to use
3. **Positive control:** *C. difficile* toxin A/B mix 2 ml ready to use
4. **Conjugate:** anti toxin A/B-HRP mix 7 ml ready to use
5. **10x Wash buffer:** 50 ml to be 10x diluted
6. **TMB – substrate:** 14 ml ready to use
7. **Stop reagent:** 7,5 ml ready to use

Accessories: adhesive plastic covers (2 units)

Materials and equipment required, but not provided:

Wash bottle	*Timer*	*Paper towels or absorbent sheets*
Discard container	*Vortex mixer*	*Microplate shaker*
Refrigerator	*Distilled water*	
Microcentrifuge	*Microplate reader*	

Preliminary preparations:

1. **Important**: All reagents must be brought to room temperature prior to their use.

2. **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.

3. **Microassay plate**: the plates are sealed in aluminum bags that need to be resealed once opened. Each strip contains 8 wells coated with antibodies specific for *C. difficile* toxin A and B. Before starting determine the number of wells to be used. Each specimen or control will employ one of these coated wells. Avoid contact with the base of the wells. Unused wells must be placed back inside of the resealable pouch with the desiccant to protect them from moisture.

Shelf life and storage:

Once opened, the kit is stable for at least 6 month when stored properly at 2-8 °C.

Precautions:

1. Reagents from different kits should not be mixed. Do not use a kit past the expiration date.

2. Reagents should be brought to room temperature before use.

3. Handle specimens and used microwells as if capable of transmitting infectious agents. Wear gloves when doing the test.

4. The substrate is sensitive to light and should be protected from direct sunlight or UV sources.

5. The positive control confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical cut-off.

Handling of fecal specimens:

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 specimen that have been centrifuged 5 min by 2500 x g to remove any particulate matter. If the tgcBIOMICS *C. difficile* GDH ELISA (Product Code: TGC-E003-1) has been used previously for primary screening, an aliquot of the same supernatant could be used and must not be prepared again.

Note: If overnight storage of the diluted sample is desirable, the storage should be done at -20°C. Otherwise, in rare cases the test could lead to false positive results.

Test procedure:

1. Add 100 µl of the prepared specimen or the toxin A/B positive control mix into each single well. As negative control use 100 µl of the dilution buffer.

2. Transfer 50 µl of the anti toxin A/B-HRP conjugate to each well.

3. Incubate the wells for 45 min at RT on a microplate shaker at 700 rpm.
   If no shaker or only one with lower speed is available, the incubation time should be extended to 60 min.

   Note: To prevent evaporation loss of liquid, the wells should be covered with plastic adhesive.

4. Wash each well with 200 µl wash buffer. Fill the wells, then shake the wash solution out of the wells into a discard pan. Slap the inverted plate on a dry paper towel.

   Note: If an automated washing system is used 300 µl Wash buffer per well are recommended.

5. Repeat step 4. four times. After washing, completely remove any residual liquid in the wells by striking the plate onto a dry paper towel until no liquid comes out.

6. Add 100 µl substrate to each well and gently tap to mix.

7. Incubate for 10 min at RT.

8. Stop the color development by adding 50 µl stop reagent to each well.

9. The addition of the stop solution converts the blue color to a yellow color that may be quantitated by measuring the optical density at 450 nm on a microassay plate reader. If a dual wavelength reader is used, blank against air at 620 nm and read at 450 nm.

Interpretation of results:

Measurement is at 450 nm and 620 nm:

- 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 $OD_{450\text{-}620}$ background should be below 0.100.

Positive control:

- The $OD_{450\text{-}620}$ of the positive control should be >1.00.

Cut off value:

- test reaction > background reaction + 0.100

For example, with a negative control value of $OD_{450\text{-}620}$ 0.030 the cut off is 0.130.

Sensitivity of the assay:

- TcdA: 0,5 ng/ml
- TcdB: 1,0 ng/ml