Datasheet for ABIN1098189

Clostridium Difficile Toxin A Or B ELISA Kit

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Overview

Quantity:	96 tests
Target:	Clostridium Difficile Toxin A Or B
Reactivity:	Clostridium difficile
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	ELISA for the separate detection of Clostridium difficile Toxin A OR Toxin B in suspensions
Sample Type:	Fecal
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	0.5 ng/mL
Components:	• 1x ELISA Plate 12x8 stripes • 1 x 50 mL dilution buffer • 1 x 2.0 mL Standard control Toxin A&B • 1 x 7 mL conjugate • 1 x 30 mL 10x washing buffer • 1 x 14 mL substrate • 1 x 7.5 mL stop solution with 0.5 M H2SO4 • 1x Manual

Target Details

Target:	Clostridium Difficile Toxin A Or B
Background:	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 antitoxin antibodies.

Application Details

Application Notes:	Sensitivity of the test is 0,5 ng/ml for TcdA as well as for TcdB.
Plate:	Pre-coated
Protocol:	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.
Sample Preparation:	• Important notes before starting:

- If the ELISA procedure should follow the one step protocol (see "Assay Procedure"), 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 BHRP 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).

- Sample Preparation:
  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 cm^2 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 specimen 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.

Assay Procedure:

- **Section 1** (Choose between one or two step protocol, then continue with section 2 of protocol)
  - **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. 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. Continue with section 2 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

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buffer) to each well to detect toxin A or toxin B.
5. Incubate for 30 min at 37 °C.
6. Continue with section 2 of the protocol.

- **Section 2**

1. 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.
2. Thereafter add 100 µl substrate to each well.
3. Incubate for 15 min at RT.
4. The color development will be stopped by adding 50 µl Stop solution to each well.
5. Measurement of the extinction will be done with a microtiter plate photometer at 450 nm versus 620 nm.

Calculation of Results:	• **Read out:** 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 OD450-OD620. • **Negative control:** The OD450-620 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:** Test reaction > 3 fold background reaction. For example, with a negative control value of OD450-620 0,030 the cut off is 0,120.
Restrictions:	For Research Use only

Handling

Handling Advice:	• 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.
Storage:	4 °C
Storage Comment:	After opening stability of plates at 4 °C will be about 6 months.
Expiry Date:	6 months

Publications

Product cited in:	Xie, Chupina Estrada, Nelson, Feng, Pothoulakis, Chesnel, Koon: "ADS024, a Bacillus velezensis strain, protects human colonic epithelial cells against C. difficile toxin-mediated apoptosis." in: **Frontiers in microbiology**, Vol. 13, pp. 1072534, (2022) (PubMed).

Publications

Zhang, Shaikh, Ferey, Wankhade, Chintapalli, Higgins, Crowley, Heitmeier, Stothard, Mihi, Good, Higashiyama, Swarts, Hruz, Shankar, Tarr, DeBosch: "Lactotrehalose, an Analog of Trehalose, Increases Energy Metabolism Without Promoting Clostridioides difficile Infection in Mice." in: **Gastroenterology**, Vol. 158, Issue 5, pp. 1402-1416.e2, (2020) (PubMed).

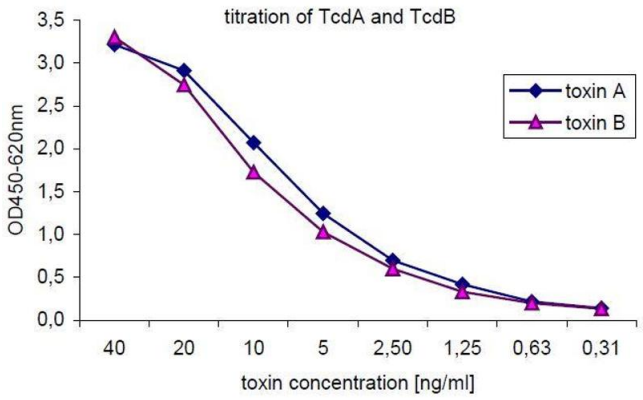
Thabit, Alam, Khaleduzzaman, Garey, Nicolau: "A pilot study to assess bacterial and toxin reduction in patients with Clostridium difficile infection given fidaxomicin or vancomycin." in: **Annals of clinical microbiology and antimicrobials**, Vol. 15, pp. 22, (2016) (PubMed).

Endres, Bassères, Khaleduzzaman, Alam, Chesnel, Garey: "Evaluating the Effects of Surotomycin Treatment on Clostridium difficile Toxin A and B Production, Immune Response, and Morphological Changes." in: **Antimicrobial agents and chemotherapy**, Vol. 60, Issue 6, pp. 3519-23, (2016) (PubMed).

Bassères, Endres, Khaleduzzaman, Miraftabi, Alam, Vickers, Garey: "Impact on toxin production and cell morphology in Clostridium difficile by ridinilazole (SMT19969), a novel treatment for C. difficile infection." in: **The Journal of antimicrobial chemotherapy**, Vol. 71, Issue 5, pp. 1245-51, (2016) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1. 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 its recommended to assay probes and standards in one reading.