Short Instruction Manual of the Assay

ELISA for the detection of Clostridium difficile Toxin A antibodies

The ELISA was developed to routinely detect the antibody response of sera from mice, hamsters and human. Titration of serially diluted sera will give a measure of the strength of the immune response. For human sera their relative IgG and IgA content can be determined. The assay is

for research use only!

Attention: The assay is available as a kit for the detection of antibodies of the following species

a. Human subtype: IgG, IgA
b. Hamster IgG

c. Mouse IgG

The content of the kit will be according to the customer’s order, i.e. it may vary.

I. Content list:

Some reagents supplied by tgcBIOMICS will depend customers needs (on the antibody species to be detected). The designation of the assay can be checked on the cover etiquette of the assay box!

1. **ELISA plate** coated with Clostridium difficile Toxin A   storage at 2 – 8 °C

2. **Positive Control**: antibody against Clostridium difficile Toxin A (ready to use)
   a. human IgG antibody 1.5 ml  storage at 2 - 8°C
   human IgA antibody 1.5 ml  storage at 2 - 8°C
   b. hamster IgG antibody (on special request)  storage at 2 - 8°C
   c. mouse IgG antibodies 1.5 ml  storage at 2 - 8°C

3. **Conjugate 10x concentrated** - peroxidase conjugated anti species antibody:
   a. detection of human antibodies:
      1: anti human IgG antibody 0.75 ml  storage at 2 - 8 °C
      2: anti human IgA antibody 0.75 ml  storage at 2 - 8 °C
   b. detection of hamster antibodies
      anti hamster IgG antibody on request only  storage at 2 - 8°C
   c. detection of mouse antibodies
      anti mouse IgG antibody 0.75 ml  storage at 2 - 8°C

4. **Dilution Buffer**
   50 ml for use in the assay (ready to use)   storage at 2 - 8 °C

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5. **10x Washing Buffer**
   30 ml to be used in the assay (be aware of the 10x concentration!) storage at 2 - 8 °C

6. **TMB - Substrate**
   14 ml ready to use storage at 2 - 8 °C

7. **Stop reagent**:
   7,5 ml ready to use storage at 2 - 8 °C

II. **To be prepared prior to testing:**

1. Prepare 1x Wash Solution: 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 aqua dest. Store your diluted 1x Wash Solution between 2°C and 8°C to avoid growth of contaminating microbes. Preparation of aliquots of the Wash Buffer is done accordingly.

2. Microtitre plate preparation: the plates are sealed in aluminum bags and once opened should be resealed with the snap closing. Once opened stability of the plate at 4°C is about 4-6 months. The plates can be used as broken "single wells" or as single strips. Each strip contains 8 wells coated with Clostridium difficile Toxin A

   Prior to testing you need to determine the number of wells for your assaying. Do not contaminate the wells with your fingers. Assay wells not used should immediately be returned to the bag and carefully resealed with desiccant.

III. **Test Procedure**

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

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

1. Pipette 100 µl of the specimen (serum or antibody dilution) diluted in Dilutions Buffer into each single well. As positive control use 100 µl of the supplied control. For the 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 washing, completely remove any residual liquid by striking the plate (wells) onto a dry paper.

4. Pipette 100µl of the conjugate to detect antibodies against Toxin A

5. Incubate for 60 min at 37°C.

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

7. Thereafter add 100µl substrate to each well

8. Incubate for 20 min at RT.

9. The color development will be stopped by adding 50µl of the stop solution to each well.

10. Measurement of the color will be by a spectrophotometer at 450 nm versus 620 nm.

IV. **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 620nm 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 value:**
   - The cut off of the assay is at OD$_{450-620}$ 0,3.
V. Typical results:

- Typical results of an antibody titration of hospitalized patients is given in the diagram attached.

The titration started with a 1:100 pre-dilution of the sera and was done in 2-fold steps. As can be seen the IgG-titer of the serum of the patient 1 is > 1:3200

VI. Standard configuration of an assay:

- For internal quality control, negative and positive standards should be run in parallel to each experiment. Except for hamster antibody detection, the positive controls are routinely available and could be ordered separately from the kit if necessary.
- To avoid background reactions predilution of the sera in the range of 1:100 (followed by further dilution in 2-fold steps) is recommended.

Interpretation of results of six sera from hospitalized patients:

- pat-1: IgG pos; IgA pos
- pat-2: IgG pos; IgA neg
- pat-3: IgG pos; IgA pos
- pat-4: IgG pos; IgA neg
- pat-5: IgG neg; IgA neg
- pat-6: IgG neg; IgA neg