ELISA kit for the identification of *Naegleria fowleri*

Kit for 96 determinations
(6 x 16 wells)

INSTRUCTIONS FOR USE

Products references: ENF-B16/6

For research invitro use only - All reagents must be stored at 4-8°C

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PRINCIPLE

Naegleria fowleri is a free-living amoeba that causes Primary Amoeba Meningo-Encephalitis (PAME), a rare but fatal disease in human. *Naegleria fowleri* has been identified worldwide, especially in warm waters.

The ELISA kit is dedicated to the identification of *Naegleria fowleri* after growth on agar-plate or liquid medium.
The principle of the kit is as follows:
The microtitration wells of the strips are sensitized with a monoclonal antibody (MAb) specific to an antigenic determinant of the amoeba *Naegleria fowleri* (Nf).
Once the amoeba is captured by the coated Mab, a second biotin-labelled antibody to Nf is allowed to react with the immobilized amoeba. A streptavidin-peroxidase complex reacts with the biotin molecules of the labelled antibody.
The peroxidase activity is detected by using a substrate converted into a coloured substance, the concentration of the latter being monitored using a spectrophotometer.

CONTENT OF KIT

All reagents must be stored between 4 and 8°C.

- ***Strip Nf 16 wells*** : 6 vacuum-packed strips of 16 wells sensitized with anti-*Naegleria fowleri* monoclonal antibody
- ***Wash solution 10x*** : Ten fold concentrated wash solution (10 x PBS) : 1x15 ml vial
- ***Positive control*** : heat-inactivated lysate of *Naegleria fowleri*: 1x1.3 ml vial
- ***Control diluant*** : 1x3 ml vial
- ***Labelled antibody*** : ready-to-use solution of biotinylated antibody to *Naegleria fowleri* : 1x11.5 ml vial
- ***Peroxidase conjugate*** : ready-to-use solution of Streptavidin labelled to horse radish peroxidase : 1x11.5 ml vial
- ***Substrate solution*** : solution of hydrogen peroxide (H_2O_2) : 1x12.5 ml (smoked glass) vial.
- ***Chromogen tablets*** : ortho-phenyl diamine (OPD) : 6x2 mg disks

PROCEDURE

The amoeba samples are individually tested.

The procedure described below relates to samples derived from agar plate cultures.
For other types of samples, it is not always necessary to use the 10x wash solution for buffering.

All reagents must be brought to room temperature before use.

1 - Application of samples

Remove squares of agar and place them in a test tube containing 500 µl of dionized water.
Buffer the sample using 55 µl of wash solution 10x (concentrated 10x PBS).
Remove 100 µl of each sample and place them in a microtitration well.

Place undiluted **positive control** (100 µl) in well A1, then place 1:2, 1:4 and 1:8 dilutions in wells B1, C1 and D1 respectively.
Use the control diluant for making the dilutions.

The negative control or optical blank (generally the last well) contains none of the reagents mentioned below. At each step, substitute 100 µl of control diluant for the reagent concerned.
Continue in this way up until step 6.
Carry out detection (7) as indicated in the leaflet.
Shake the plate whilst incubating for 1 hour at room temperature

2 - Washes

Dilute the wash solution 10x 1:10 using deionized water and use to wash 3 times with 150 µl per wells.

3 - Application of the labelled antibody

Place 100 µl of labelled antibody solution (ready-to-use) in each well.
Incubate the plate for 30 minutes at room temperature.

4 - Washes

Dilute the 10x wash solution 1:10 using deionized water and use to wash 3 times with 150 µl per wells.

5 - Application of the peroxidase conjugate

Place 100 µl of peroxidase conjugate (ready-to-use solution) in each well. Incubate the plate for 30 minutes at room temperature.

6 - Washes

Dilute the 10x wash solution 1:10 using deionized water and use to wash 3 times with 150 µl per wells.

7 - Detection of enzyme activity

Remove extemporaneously the 2 mg disk of OPD carefully (use gloves or tweezers) - Avoid all contact with the skin or eyes.

For each strip :
- completely dissolve the 2 mg disk of OPD in 2 ml of substrate solution.
- place 100 µl of the mixture in each well.
- protect the strip from light and shake for 10 minutes at room temperature.

Ortho-phenyl diamine, a potentially carcinogenic product classified as "toxic", is used in the system for detecting enzyme activity.
The manufacturer adds the following comments :
"Toxic. May cause cancer.
Harmful, by inhalation, in contact with skin and if swallowed.
Irritating with eyes, respiratory system and skin.
May cause sensitization by inhalation and skin contact.
Target organ(s) : bladder, liver".

8 - Reading

Use a microplate reader at 450 nm to read the colour intensity.
Use air or one of the wells containing 100 µl of dionized water for setting the optical zero.

INTERPRETATION OF RESULTS

A sample is considered to be positive if the optical density (OD) read off at 450 nm is greater than 0.2.

For an OD between 0.1 and 0.2, it is recommended that the test be repeated using a 1:2 dilution of the sample.

To interpret the results quantitatively, plot a curve of OD as a function concentration using values from the *Naegleria fowleri* range.
From the curve, read off the concentration of the sample under test.

PRECAUTIONS

***Naegleria fowleri* is a pathogen which causes primary amoebic meningoencephalitis (PAME) that can be fatal in man. The usual precautions must be strictly respected when handling such organisms. It is strongly recommended that masks, gloves and protective goggles be worn.**

The positive control consists of a suspension of heat inactivated *Naegleria fowleri* from agar-plate culture. Although no growth on agar-plate has been observed, it is recommended to consider this sample as potentially pathogenic.

Reagents must be stored between 4°C and 8°C.
Do not draw up reagents by mouth.
Use only scrupulously clean glassware.
Follow the instructions for handling, and the incubation times and temperatures. This is essential in ensuring accurate results.

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