CREATIVE BIOMART®
Assay Kit

Cryptosporidium Water Testing Kit

Product Information

Common Name

Cryptosporidium

Cat.No. Kit-2442

Product Overview

The kit is designed to detect the cyst and oocyst stages of these parasites in particulates isolated from water and other environmental samples utilizing the principle of direct immunofluorescence.

Storage

Store at 4° C. DO NOT FREEZE.
Kit-2442-1X reagent, C101, D101, and M101 are light sensitive.

Kit Components

Kit-2442-1X: 1 dropper vial containing 3.5 mL working dilution (1x) reagent
PC101: 1 glass vial containing 1 mL positive control
WB101: 1 screw cap bottle containing 50 mL 1x Wash Buffer
C101: 1 dropper vial containing 3.5 mL counterstain
D101: 1 microtube containing 0.4 mL DAPI, 5000X in methanol
M101: 1 dropper vial containing 3.5 mL Mounting Medium
S100-2: 1 box of two-well Slides, 40/box

Technical Notes

1. Test Time: Approximately 35 – 40 minutes after the sample is dried to the well slide and without methanol fixation step. (Approximately 1.0 hr when performing methanol fixation.)
2. Kit-2442-1X, antibody, FL, reagent will stain both viable (live) and non-viable (dead) cells. It will stain cysts and oocysts preserved by gamma irradiation or suspended in formalin.
3. When making a positive control slide using PC101, mix the contents of the vial prior to use. Vortex the vial for 20 seconds immediately before use. Note: The number of organisms in PC101 is not exact and should not be used for sample recovery estimation.
4. Prepared slides (mounted with M101, mounting medium) may be kept in a refrigerator/protected

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from light and viewed repeatedly for 6 months or longer. DAPI staining may fade.
5. Steps 3 & 4 can be performed after steps 5 & 6, that is, DAPI may be applied to the sample well either before staining with antibody or after.
6. If DAPI staining appears faint, the reaction time may be increased from 1 minute to 4 minutes. Another option is to increase the concentration to 1 ug/mL. To dilute DAPI to 1 ug/mL, add 2.5 uL D101 to 5 mL PBS or 25 uL DAPI to 50 mL PBS. If DAPI staining continues to be faint, the concentration can be increased further to 2 ug/mL. To dulute to 2 ug/mL, add 5 uL D101 to 5 mL PBS or 50uL D101 to 50 mL of PBS.
7.One resource available to help distinguish between Giardia cysts, Cryptosporidium oocysts and possible cross-reactors can be found on the US EPA website. The US EPA has developed training modules for the Long Term 2 (LT2) Enhanced Surface Water treatment Rule. These training modules were developed to assist analysts in the detection and identification of Giardia and Cryptosporidium.

Preparation

1. Prepare environmental sample(s) to be applied to well slide.
2. Dilute DAPI to a 1X working dilution.
• Add 1 uL D101 to 5 mL of PBS (phosphate-buffered saline solution, pH 7.4). Alternatively, 10 uL may be diluted in 50 mL PBS. Mix by inversion. Prepare working dilution daily. Discard any unused 1X solution.

Assay Protocol

1. Isolated water particulates should be air-dried onto a well of a pre-treated slide, using a stream of warm (not hot) air; alternatively, a slide-warmer may be used. Do not allow the slide to become hot to the touch. Samples must be completely dry before continuing to step 2. (Drying time: Approximately 15 – 30 minutes.)
2. A methanol fixation step may be performed at this point, however, it is not required for this reagent to bind well to oocysts. Methanol fixation may intensify DAPI staining. Methanol fixation: Apply 45-uL absolute methanol to the well of the slide. Allow the well of the slide to dry completely. (Drying time: Approximately 30 minutes.)
3. When the sample has dried completely, DAPI staining may be performed here. Add 50 uL of a

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working dilution (1X) of 4",6-diamidino-2-phenylindole (DAPI) to each sample well. Leave on sample for 1 minute at room temperature.
4. Rinse the slide free of DAPI by adding 50 – 100 uL wash buffer and leave for 1 minute. Tilt slide, long edge down, and absorb excess fluid with absorbent material placed at the edge of the slide well. Do not touch the surface of the well slide or disturb the sample.
5. Apply one drop (approximately 45 uL) of antibody reagent to the spot of dried test particulates in each well. If necessary, spread the drop with applicator stick or glass rod, being careful not to contact the surface of the slide.
6. Incubate the slide in a humid chamber at room temperature for at least 25 minutes. If using a 37° C incubator, incubate for 25 minutes. Longer incubation periods are OK.
7. Rinse the slide free of antibody reagent by adding 50 – 100 uL wash buffer and leave for 1 minute. Tilt slide, long edge down, and absorb excess fluid with absorbent material placed at the edge of the slide well. Do not touch the surface of the well slide or disturb the sample.
8. Non-specific background fluorescence may be reduced, and a reddish background added to enhance contrast, by the use of counterstain at this stage. Apply 1 drop of counterstain per well. Incubate for 1 minute at room temperature.
9. Rinse the slide free of counterstain by adding 50 – 100 uL wash buffer and leave for 1 minute. Tilt slide, long edge down, and absorb excess fluid with absorbent material placed at the edge of the slide well. Do not touch the surface of the well slide or disturb the sample.
10. The slide should be partially-to-completely air dried on a slant and then mounted with one drop (~ 45 uL) of mounting medium. Apply cover glass and view.