

Cryptosporidium Water Testing Kit

Cat. No. Kit-2442 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Cryptosporidium Water Testing Kit designed to detect the oocyst stage of Cryptosporidium parvum in particulates isolated from water and other environmental samples utilizing the principle of direct immunofluorescence.
Description	Cryptosporidium is a genus of apicomplexan parasitic alveolates that can cause a respiratory and gastrointestinal illness (cryptosporidiosis) that primarily involves watery diarrhea (intestinal cryptosporidiosis) with or without a persistent cough (respiratory cryptosporidiosis) in both immunocompetent and immunodeficient humans.
Quality Control Test	Titration of antibody reactivity by immunofluorescence with freshly prepared C. parvum oocysts on specially treated slides. F/P ratio is measured spectrophotometrically.
Storage	Store at 4-8 centigrade
Size	1kit (75+ assays)
Kit Components	A: 1 dropper vial containing 3.5 mL working dilution (1x) reagent B: 1 glass vial containing 1 mL positive control C: 1 screw cap bottle containing 50 mL 1x Wash Buffer D: 1 dropper vial containing 3.5 mL counterstain E: 1 microtube containing 0.4 mL DAPI, 5000X in methanol F: 1 dropper vial containing 3.5 mL Medium B

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G: 1 box of two-well slides, 40/box

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 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 Cryptosporidium 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

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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 Medium B. Apply cover glass and view.