A400FLR-20X. Crypt-a-Glo™, FL, 20x conc., Reagent Only

Fluorescein-labeled Monoclonal Antibody Reagent for Direct Immunofluorescence Detection
Of *Cryptosporidium* Oocysts in Water Samples

Crypt-a-Glo™ is EPA - approved for use in Methods 1622 and 1623

Explanation: *Cryptosporidium parvum* is a common, ubiquitous intestinal parasitic protozoan that causes gastroenteritis in man and lower animals. This organism has a reservoir of host animals and can be spread through food, water, and fomites. The *Cryptosporidium* oocyst is a nearly round, encysted, environmentally resistant organism of approximately 3-5 um in diameter, containing up to four sporozoites. The oocyst is the life cycle stage that would be present in water particulates.

Description of Products:

» The Crypt-a-Glo™ reagent is designed to detect the oocyst stage of *Cryptosporidium* utilizing the principle of direct immunofluorescence. It is not meant for detection of *Cryptosporidium* life cycle stages other than the oocyst.

» The antibody reagent included consists of a fluorescein-labeled mouse monoclonal antibody made to an oocyst wall antigenic site (epitope) of *Cryptosporidium parvum*. The reagent shows varying degrees of cross-reactivity with oocysts of other species of *Cryptosporidium*. The oocysts will appear bright apple-green when viewed under a fluorescence microscope using the appropriate filters for fluorescein. The reagent is a 20x concentrated solution and provides enough reagent for over 400 tests, *using one drop per test (approximately 45 uL per drop.)* The antibody reagent contains 0.04% w/v sodium azide as preservative and 1% bovine serum albumen as antibody stabilizer.

» Dilution/Blocking Buffer is designed for diluting concentrated formats of Waterborne, Inc. fluorescent antibodies to working (1X) dilution, for stabilizing the antibodies for long storage life, and for optimal reduction of background, i.e. non-specific binding, of the antibodies. The buffer can also be used to 'pre-block' filters before adding the antibodies.

» No-Fade™ Mounting Medium is fade retardant. Minimize exposure to light. Some yellowing may occur over time with exposure to light – this will not affect performance.

» Positive control is a mixture of *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts in a buffered mixed aldehyde solution. The concentration of this suspension is approximately 2x10e5 cysts and oocysts (each) per mL. (These numbers are not exact and should not be used for sample recovery estimation.)

Storage: Store at 4° C. DO NOT FREEZE.
A400FLR-20X reagent and M101 are light sensitive.

Kit Includes:

- A400FLR-20X: 1 glass vial containing 1.0 mL 20x concentrated reagent. This solution must be diluted to 1x before use.
- B100-20: 1 screw cap bottle containing 20 mL Dilution/Blocking (DB) Buffer
- PC101: 1 glass vial containing 1 mL positive control
- M101: 1 dropper vial containing 3.5 mL No-Fade™ Mounting Medium

Other Lab Supplies Not Included, but Available:

- B100-40: 40 mL Dilution/Blocking (D/B) Buffer
- C101: 3.5 mL BlockOut™ counterstain
- D10 1: 0.4 mL DAPI, 5000X in methanol
- M101FF: 3.5 mL No-Fade™ Mounting Mediu, Formalin-Free
- M102: 3.5 mL Elvanol No-Fade™ Mounting Medium
- S100-1-9MM: One-well (9mm) SuperStick™ Slides, 40/box
- S100-1: One-well (14mm) SuperStick™ Slides, 40/box
- S100-2: Two-well SuperStick™ Slides, 40/box
- S100-3: Three-well SuperStick™ Slides, 40/box
- WB100: 50 mL 1x SureRinse™ Wash Buffer
- WB101: 50 mL 20x SureRinse™ Wash Buffer
- PACIR: AccuSpike™-IR, G/C Quality Control Standard (PACIR3, PACIR6, PACIR12)

Preparation

1. Prepare environmental sample(s) to be applied to well slide.

2. Dilute an aliquot of the concentrated antibody reagent 1:20 (one part up to 20 parts) with DB buffer to the required volume of 1x working dilution. For example, if 1 mL of reagent is needed, dilute 50 uL of 20x solution with 950 uL of DB buffer. If 20 mL of reagent is needed, dilute 1 mL 20x solution with 19 mL DB buffer.

Contact us by email for MSDS or Certificate of Analysis/QC Report.
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Instructions for Use:

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 cysts and 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 SureRinse™ wash buffer, or equivalent, 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 Crypt-a-Glo™ 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 SureRinse™ wash buffer, or equivalent, 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 BlockOut™ 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 SureRinse™ wash buffer, or equivalent, 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 No-Fade™ mounting medium. Apply cover glass and view.

Other Information, Tips & Troubleshooting

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. A400FLR-20X, Crypt-a-Glo™ Direct, 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, No-Fade™ mounting medium) may be kept in a refrigerator/protected 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 Crypt-a-Glo™ 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. 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*.
   They can be found at: www.epa.gov/safewater/lt2/training/index.html.

For assistance, technical questions, or to inquire about other Waterborne, Inc. products, please call, FAX, or email us. Also, please visit our website at: www.waterborneinc.com.

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