Catalog Nr: A100FLR-20X

Aqua-Glo™ G/C Direct, FL, 20x concentration, Reagent Only Kit

Fluorescein-labeled Monoclonal Antibody Reagent for Simultaneous Direct Immunofluorescence Detection of *Giardia* Cysts and *Cryptosporidium* Oocysts in Water Samples

Aqua-Glo™ G/C is EPA - approved for use in Methods 1622 and 1623.

Explanation: *Giardia lamblia* and *Cryptosporidium parvum* are common, ubiquitous intestinal parasitic protozoa that cause gastroenteritis in man and lower animals. Both organisms have a reservoir of host animals and can be spread through fecal contamination of food, water, and fomites. The *Cryptosporidium* oocyst is a nearly round encysted organism of approximately 3-5 um in diameter, while the *Giardia* cyst is oval-shaped and measures approximately 8-13 um in length and 7-10 um in width.

Description of Products

» The Aqua-Glo™ 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.

» The antibody reagent consists of a mixture of fluorescein-labeled mouse monoclonal antibodies made to cyst and oocyst outer wall antigenic sites (epitopes) of *Giardia lamblia* and *Cryptosporidium parvum*. This reagent is genus-specific and will bind only to the cysts and oocysts of these two parasites if they are present. The reagent shows varying degrees of cross-reactivity with cysts and oocysts of other species of *Giardia* and *Cryptosporidium*. The cysts and/or oocysts will appear bright apple green when viewed under a fluorescence microscope using the appropriate filters for fluorescein. This antibody cross-reacts with some species of algae.

» 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 mixed aldehyde buffer. 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.

A100FLR-20X reagent and M101 are light sensitive.

Kit Includes

- A100FLR-20X: 1 glass vial containing 1.0 mL 20x concentrated reagent solution
  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 Medium, 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: 1 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.
Email: contact@waterborneinc.com

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 Aqua-Glo™ G/C 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.
    Note: M101FF, No-Fade™ mounting medium without formalin, may be used in the place of M101 for laboratories planning to perform PCR genotyping on *Cryptosporidium* positive slides.

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. A100FLR-20X, Aqua-Glo™ G/C 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 Aqua-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. 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 e-mail us. Also, please visit our website at www.waterborneinc.com.

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