**Catalog Nr: A100FLR-1X**

# Aqua-Glo™ G/C Direct, FL, 1x 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 objects. 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.

» 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.

» DAPI (4,6-diamidino-2-phenylindole) is prepared at 2mg/mL in methanol (5000X stock solution). The volume is 0.4 mL. A 1X solution can be prepared by diluting 1 uL of DAPI in 5 mL PBS (phosphate-buffered saline solution, pH 7.4) or 10 uL diluted in 50 mL PBS. DAPI binds to DNA, fluorescing blue using a UV filter setting. Minimize exposure to light.

» SureRinse™ Wash Buffer is a 1X working dilution buffer provided for the rinse processes. This buffer needs no dilution prior to use.

» 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-1X reagent is light sensitive.

**Kit Includes**

- A100FLR-1X: 2 bottles containing 3.5 mL working dilution (1x) reagent each
- PC101: 1 glass vial containing 1 mL positive control
- WB101: SureRinse™ Wash Buffer, 50 mL
- D101: DAPI, 400 uL of 5000X solution in methanol
- M101: 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
- 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 20x SureRinse™ Wash Buffer
- PACIR: AccuSpike™-IR, G/C Quality Control Standard (PACIR3, PACIR6, PACIR12)

**Lab Supplies Required, but Not Included:**

- Pipettes
- Coverslips
- Membranes
- Membrane Manifold and Vacuum Source or
- Vacuum Source with a Liquid Trap

## Preparation

1. Prepare environmental sample(s) to be applied to membrane.

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.

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 retained in a 1.5 mL microtube.

2. The sample may be stained in suspension or directly on the membrane. This method should be followed to stain the organisms directly on the membrane.

3. Apply the vacuum source to the membrane manifold or membrane at < 1 in Hg. Rinse the membrane with 1 mL deionized water.

4. Apply the sample to the membrane using a pipet. Rinse the membrane, again, with 1 mL deionized water.

5. Add 200 uL deionized water with 0.01% Tween 20 to the microtube. Close the cap and vortex the microtube for 30 seconds to rinse the tube. Apply the rinsate to the membrane using a pipet. Rinse the membrane, again, with 1 mL deionized water.

6. Turn the vacuum source off.

7. Add 100 uL of a working dilution (1X) of 4",6-diamidino-2-phenylindole (DAPI) to membrane. Leave on sample for 1 minute.

8. Remove the DAPI by turning on the vacuum source at < 1 in Hg. Once the DAPI has been removed, turn the vacuum off. Rinse the membrane by applying 100 uL SureRinse™ wash buffer and leave for 1 minute. Turn the vacuum on, again, to remove the wash buffer.

9. Turn the vacuum source off. Optionally: The tubing to the vacuum source may be disconnected to eliminate any residual vacuum pressure.

10. Apply 2 drops (~90 uL) of Aqua-Glo™ G/C antibody reagent to the membrane. Leave the reagent on the membrane for 25 minutes.

11. (If the tubing was disconnected, re-connect it.) Remove the reagent by turning on the vacuum source at < 1 in Hg. Once the reagent has been removed, turn the vacuum off. Rinse the membrane by applying 100 uL SureRinse™ wash buffer and leave for 1 minute. Turn the vacuum on, again, to remove the wash buffer.

12. Turn the vacuum source off.

13. Apply 10 uL No-Fade™ Mounting Medium to a slide. Transfer the membrane to the slide with the mounting medium already applied.

14. Apply 10 uL No-Fade™ Mounting Medium to the membrane. Cover the membrane with a cover glass.

## Other Information, Tips & Troubleshooting

1. Test Time: Approximately 35 – 40 minutes.

2. A100FLR-1X, 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 membrane 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.

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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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