A100FLACK. Aqua-Glo™ G/C Direct Comprehensive Kit with AccuSpike™-IR

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

Aqua-Glo ™ G/C and AccuSpike ™-IR are EPA - approved for use in Methods 1622, 1623, and 1623.1.

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.

» BlockOut™ Counterstain contains Evans Blue. It binds nonspecifically, fluorescing red using a fluorescein filter setting, enhancing contrast with the apple-green fluorescence of the specific antibody reaction.

» 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.

» SuperStick™ Slides are chemically treated to increase adhesion of cysts, oocysts, spores, and other cells. The wells measure 15 mm in diameter. Each slide has a green, Teflon-coated section that is hydrophobic to contain the sample within the well. Each slide also has a frosted area at one end for writing with pencil or marker. Packaged forty slides per box.

» 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.)

» AccuSpike™-IR vials (12) are designed for percent recovery determination with matrix (environmental) and reagent water samples. Each 2.0 mL polypropylene vial contains a count, by flow-cytometry cell sorting, of 100 each of *G. lamblia* cysts and *C. parvum* oocysts. The organisms are suspended in 0.75 mL de-ionized water with 0.01% Tween 20, and have been inactivated by gamma (γ) irradiation. The internal structures of the cysts and oocysts are intact and easily identified, and the cells are fully reactive with antibody-coated immunomagnetic particles and fluorescent antibodies. Although they are non-infectious, as proven by mouse inoculation and in vitro monolayer inoculation experiments, proper laboratory procedures and personal protective equipment are recommended as per US EPA Methods 1622, 1623, and 1623.1.

Expiration Dates: All components of this kit, except AccuSpike™-IR, have an expiration of 18 months after the date of production. AccuSpike™-IR vials have an expiration of only 12 weeks after the date of production. *Each component is clearly marked with an expiration date*

Storage: Store at 4° C. DO NOT FREEZE.

A100FLR-1X reagent, C101, D101, and M101 are light sensitive.

Kit Includes

- A100FLR-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 SureRinse™ Wash Buffer
- C101: 1 dropper vial containing 3.5 mL BlockOut™ counterstain
- D101: 1 microtube containing 0.4 mL DAPI, 5000X in methanol
- M101: 1 dropper vial containing 3.5 mL No-Fade™ Mounting Medium
- S100-2: 1 box of two-well SuperStick™ Slides, 40/box
- PACIR12: AccuSpike™-IR, G/C Quality Control Standard, 12 vials

Other Lab Supplies Not Included, but Available

- S100-1-9MM: One-well (9mm) SuperStick™ Slides, 40/box
- S100-1: One-well (14mm) SuperStick™ Slides, 40/box
- S100-3: Three-well SuperStick™ Slides, 40/box
- M101FF: Formalin-Free No-Fade™ Mounting Medium
- M102: 3.5 mL Elvanol No-Fade™ Mounting Medium
- WB100: 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 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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AccuSpike™ Organism Transfer Protocol:
(For transfer of organisms from AccuSpike™ vial only.)

1. Uncap the AccuSpike™-IR vial. Add **0.25 mL** EluMax™.

2. **Initial Transfer**: Re-cap vial and vortex 20 seconds. Carefully open the vial. Using a Pasteur pipet, remove the liquid from the vial and any liquid that has collected in the cap. Add this to the 10 L or more of water to be used in Methods 1622, 1623, or 1623.1. Retain the pipet for use in subsequent steps.

3. **First Rinse**: Add 0.75 mL de-ionized water to the vial, re-cap tightly, and vortex for 15 seconds.

4. Carefully open the vial, and remove the liquid from the vial as before. Add this to the water. Retain pipet for use in subsequent steps.

5. **Second Rinse**: Repeat steps 3 & 4.

6. Discard the empty vial.

7. Please see US EPA Method 1622, Method 1623, Method 1623.1, or LT2 Rule for Sample Spiking Procedure.
(Visit www.epa.gov for more information.)

Antibody Staining Instructions:

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 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 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 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 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. 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 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.

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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