Catalog Nr: A300FLK
Giardi-a-Glo™ Comprehensive Kit

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

Giardi-a-Glo™ is EPA - approved for use in Method 1623.

Explanation: *Giardia lamblia* 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 fecal contamination of food, water, and objects. The *Giardia* cyst is oval-shaped and measures approximately 8-13 um in length and 7-10 um in width.

Description of Products

» The Giardi-a-Glo™ kit is designed to detect the cyst stage of this parasite in particulates isolated from water and other environmental samples utilizing the principle of direct immunofluorescence.

» The antibody reagent consists of a fluorescein-labeled mouse monoclonal antibody made to cyst outer wall antigenic sites (epitopes) of *Giardia lamblia*. This reagent is genus-specific and will bind only to the cysts of this parasite if they are present. The reagent shows varying degrees of cross-reactivity with cysts of other species of *Giardia*. The cysts will appear bright apple green when viewed under a fluorescence microscope using the appropriate filters for fluorescein.

» 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.)

Storage: Store at 4° C. DO NOT FREEZE.
A300FLR-1X reagent, C101, D101, and M101 are light sensitive.

Kit Includes

- A300FLR-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

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: 3.5 mL No-Fade™ Mounting Mediu, Formalin-Free
- 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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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**. 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 Giardi-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 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 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. A300FLR-1X, Giardi-a-Glo™, FL, reagent will stain both viable (live) and non-viable (dead) cells. It will stain cysts 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 Giardi-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. 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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