ST101R. Cyst-a-Glo™ Reagent-Only Kit

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

Explanation: The Cyst-a-Glo™ kit is intended for use in diagnosis of human or animal infection with the parasites, *Giardia lamblia* and *Cryptosporidium parvum*, by direct immunofluorescence. The test is designed for testing thin smears of stool (fecal) specimens made on our specially treated multi-well microscope slides. The test demands access to and skill in using a fluorescence microscope.

Description of Products

» *Giardia lamblia* and *Cryptosporidium parvum* are common, ubiquitous intestinal parasitic protozoa that cause gastroenteritis in man and lower animals. Both are spread by food, water, and fomites. A range of reservoir host animals exists for both parasites. Symptoms of infection with both organisms may include diarrhea, intestinal cramping, bloating, gas, vomiting, and, occasionally, low-grade fever. Severe infection with *Cryptosporidium* is often associated with immunosuppressed conditions, including AIDS. The cellular stages that would appear in the feces of infected persons or lower animals would include, for *Cryptosporidium*, the oocyst, a nearly round encysted cell of a approximately 3-5 um in diameter; and for *Giardia*, the oval-shaped cyst and, far less commonly, the pear-shaped trophozoite. The *Giardia* cyst measures approximately 8-13 um in length and 7-10 um in width; the trophozoite, approximately 9-21 um long by 5-15 um wide.

» The Cyst-a-Glo™ kit is designed to detect the cyst and oocyst stages of these parasites. It will not detect the *Giardia* trophozoite or other life cycle stages of *Cryptosporidium*.

» The Cyst-a-Glo™ kit utilizes the principle of direct immunofluorescence. The Cyst-a-Glo™ reagent consists of a mixture of fluorescein-labeled mouse monoclonal antibodies made to cyst wall antigenic sites (epitopes) of *Giardia lamblia* and *Cryptosporidium parvum*. The reagent will bind only to the cysts and oocysts of these two parasites if they are present in the stool or feces. The cysts and/or oocysts will appear bright apple-green when viewed under a fluorescence microscope using the appropriate filters for fluorescein.

» Reagent included is a working dilution (1x) of fluorescein (FL)-labeled monoclonal antibodies made against cysts of *Giardia lamblia (= G. intestinalis*) and oocysts of *Cryptosporidium parvum*. This kit provides enough reagent for at least 75 tests, using one drop per test (approximately 45 microliters per drop). The antibody reagent contains 0.04% w/v sodium azide as preservative and 1% bovine serum albumen as antibody stabilizer. This reagent show varying degrees of cross-reactivity with cysts and oocysts of other species of *Giardia* and *Cryptosporidium*.

» Positive Control is a mixture of Giardia lamblia cysts and Cryptosporidium parvum oocysts in a buffered formaldehyde solution with fecal material. The concentration of this suspension is approximately 2x10e5 cysts and oocysts (each) per mL.

Storage: Store at 4° C. DO NOT FREEZE.
ST101R reagent is light sensitive.

Kit Includes:

- ST101R: 1 dropper vial containing 3.5 mL working dilution (1x) reagent
- PC101: 1 screw cap vial containing 1.0 mL positive control

Other Lab Supplies Not Included, but Available:

- B100-40: 40 mL Dilution/Blocking (D/B) Buffer
- C101: 3.5 mL BlockOut™ counterstain
- D101: 0.4 mL DAPI, 5000X in methanol
- M101: 3.5 mL No-Fade™ Mounting Medium
- 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
- WB101: 50 mL 1x 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.

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 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 Cyst-a-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 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. ST101R, Cyst-a-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 Cyst-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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