



A400FLK. Crypt-a-Glo™ Comprehensive Kit
Fluorescein-labeled Monoclonal Antibody Reagent for Direct Immunofluorescence Detection
of Cryptosporidium Oocysts in Water Samples
Crypt-a-Glo™ is EPA - approved for use in Methods 1622 and 1623.

Explanation: Cryptosporidium parvum is a common, ubiquitous intestinal parasitic protozoan that causes gastroenteritis in man and lower animals. It is spread through food, water, and fomites. A range of reservoir host animals exists for this parasite. Symptoms of infection 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 the oocyst, a nearly round encysted cell of approximately 3-5 um in diameter. The **Crypt-a-Glo™** kit is designed to detect the oocyst stage of this parasite. It will not detect any other life cycle stages of Cryptosporidium other than the oocyst.

Biological Principles: This kit utilizes the principle of direct immunofluorescence. The **antibody** reagent consists of a fluorescein-labeled mouse monoclonal antibody made to cyst wall antigenic sites (epitopes) of Cryptosporidium parvum. The reagent will bind only to the oocysts of this parasite if they are present. The oocyst will appear bright apple-green when viewed under a fluorescence microscope using the appropriate filters for fluorescein.

Description of Products: Reagent included is a working dilution (1x) of a fluorescein (FL)-labeled monoclonal antibody made to 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 shows varying degrees of cross-reactivity with oocysts of other species of Cryptosporidium. Counterstain contains Evan's Blue with 0.04% w/v sodium azide as preservative. Mounting Medium is fade retardant. Positive Control is a mixture of Giardia lamblia cysts and Cryptosporidium parvum oocysts in 1% formalin in PBS. The concentration of this suspension is approximately 2x10e5 cysts and oocysts (each) per mL. 20x wash buffer is a saline solution provided for the rinsing process. SuperStick™ Slides are chemically treated to increase cell adhesion. The wells measure 15 mm in diameter.

Kit Includes:

- 1 dropper vial containing 3.5 mL working dilution (1x) reagent
- 1 dropper vial containing 3.5 mL counterstain (catalog # C101)
- 1 dropper vial containing 3.5 mL No-Fade™ Mounting Medium (catalog # M101)
- 1 screw cap vial containing 1.5 mL positive control
- 1 glass bottle containing 50 mL 20x wash buffer
- 1 box of two-well SuperStick™ Slides, 40/box (catalog # S100-2)

Other Lab Supplies Not Included, but Available:

- S100-1: One-well SuperStick™ Slides, 40/box
- S100-3: Three-well SuperStick™ Slides, 40/box
- M102: 3.5 mL Elvanol No-Fade™ Mounting Medium

Storage: Store kit at approximately 4° C. DO NOT FREEZE!

Instructions for Use:

1. Thin smears or spots of the specimen should be air-dried onto a well of a 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.
2. When the sample has dried, a methanol fixation step may be performed at this point; however, methanol fixation is not required for this reagent to bind well to cysts and oocysts nor will it interfere with the antigen-antibody reaction.
3. When the sample has dried **completely**, apply one drop (approximately 45 uL) of **Crypt-a-Glo™** antibody reagent to the dried fecal smear or spot in each well. If necessary, spread the drop with applicator stick or glass rod, being careful not to contact the surface of the slide.
4. Incubate the slide in a humid chamber at room temperature for at least **40 minutes**. If using a 37° C incubator, incubate for 30 minutes. Longer incubation periods are OK.
5. Rinse the slide free of antibody reagent by soaking the slide in a Coplin jar, beaker, or other vessel for approximately 60 seconds in 1x saline wash solution or in "PBS" (phosphate-buffered saline).
6. Non-specific background fluorescence may be reduced, and a reddish background fluorescence added to enhance contrast, by the use of counterstain at this stage. Apply 1 drop of counterstain per well. (Incubate for one minute. Follow with a one-minute rinse with saline or PBS. Longer incubations are OK.)
7. Following a rinse, the slide should be partially to completely air-dried on a slant and mounted with one drop per well of the mounting medium and either one 22x50 mm coverslip or two 22x22 mm coverslips.

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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