A600FLR-20X. Sporo-Glo™ Reagent-Only Kit

Fluorescein-labeled Antibody Reagent for Direct Immunofluorescence Detection
Of Intracellular Reproductive Stages of *Cryptosporidium parvum* (FDM assay, etc.)

Explanation: The Sporo-Glo™ kit is intended for use in conjunction with the Focus Detection Method (FDM)[1] for evaluating the viability of *Cryptosporidium parvum* oocysts in sterile slide chambers with mammalian monolayers. The test demands access to and skill in using a fluorescence microscope. In this test, oocysts are inoculated into sterile slide chambers containng a mammalian cell monolayer. This kit is designed for detection of internal dividing stages of *Cryptosporidium parvum* labeled within the monolayer cells by immunofluorescence.

Description of Products:

» The Sporo-Glo™ kit utilizes the principle of direct immunofluorescence. The reagent consists of a fluorescein-labeled rat anti- *Cryptosporidium parvum* sporozoite polyclonal antibody. The reagent will bind to sporozoites, merozoites, and all other intracellular reproductive stages; the specimens will appear bright apple-green when viewed under a fluorescence microscope using the appropriate filters for fluorescein.

» The reagent included is a concentrated solution (20x) of fluorescein (FL)-labeled polyclonal rat IgG antibody made against sporozoites of the Iowa bovine isolate of *Cryptosporidium parvum*. The antibody reagent contains 0.04% w/v sodium azide as preservative and 1% bovine serum albumen as antibody stabilizer. This reagent reacts more strongly with the intracellular dividing stages than it does with sporozoites. There is some degree of cross-reactivity of this reagent with oocyst walls.

» Dilution/Blocking Buffer is designed for diluting concentrated formats of Waterborne, Inc. fluorescent antibodies to working (1X) dilution, for stabilizing the antibodies for long storage life, and for optimal reduction of background, i.e. non-specific binding, of the antibodies. The buffer can also be used to 'pre-block' filters before adding the antibodies.

» 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.

» No positive control is provided in this kit. The recommended positive control is a freshly infected culture monolayer.

Storage: Store at 4° C. DO NOT FREEZE.
A600FLR-20X reagent and M101 are light sensitive.

Kit Includes:

- A600FLR-20X: 1 glass vial containing 1.0 mL 20x concentrated reagent solution This solution must be diluted to 1x before use
- B100-20: 1 screw cap bottle containing 20 mL Dilution/Blocking (DB) Buffer
- M101: 1 dropper bottle vial containing 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
- D10 1: 0.4 mL DAPI, 5000X in methanol
- M102: 3.5 mL Elvanol No-Fade™ Mounting Medium
- M101FF: 3.5 mL No-Fade™ Mounting Mediu, Formalin-Free
- 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 1x SureRinse™ Wash Buffer
- WB101: 50 mL 20x SureRinse™ Wash Buffer
- PACIR: AccuSpike™-IR, G/C Quality Control Standard (PACIR3, PACIR6, PACIR12)

Preparation

1. Dilute an aliquot of the concentrated antibody reagent 1:20 (one part up to 20 parts) with DB buffer to the required volume of 1x working dilution. For example, if 1 mL of reagent is needed, dilute 50 uL of 20x solution with 950 uL of DB buffer. If 20 mL of reagent is needed, dilute 1 mL 20x solution with 19 mL DB buffer.

Contact us by email for MSDS or Certificate of Analysis/QC Report.
Email: contact@waterborneinc.com

Waterborne, Inc., Clinical and Environmental Parasitology Products, 6045 Hurst Street, New Orleans, LA 70118 USA
Tel/Fax: 504-895-3338. Toll-free: 866-895-3338, Email: custserv@waterborneinc.com, Website: www.waterborneinc.com

Instructions for Use:

1. Rinse the monolayer and treat with methanol as suggested in the Focus Detection Method. Alternatively, cover the monolayer with enough methanol and allow to stand for 8 minutes. Drain methanol out of each well.

2. Add ~250uL of DB Buffer included in the kit to monolayer and allow to stand for 30 minutes. Drain buffer out of each well.

3. Apply working 1x dilution of antibody reagent to completely cover the monolayer. Incubate the slide in a humid chamber at room temperature for at least 45 minutes. Longer incubation periods are OK.

4. Drain the antibody reagent out of each well. Rinse the slide of antibody reagent by applying "PBS" (phosphate-buffered saline) drop wise to cover the monolayer. Allow to sit for 3 minutes. Drain the PBS out of each well.

5. NOTE: Non-specific background fluorescence may be reduced, and a reddish background fluorescence added to enhance contrast, by the use of Waterborne™, counterstain (C101) at this stage. (1-minute reaction time, followed by a 1-minute rinse in saline or PBS.)

6. Remove chamber walls. The slide should next be mounted with one drop (~45 uL) of No-Fade™ Mounting Medium and covered with a coverslip.

Other Information, Tips & Troubleshooting

1. Test Time: Approximately 90 – 95 minutes.

2. 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.

For assistance, technical questions, or to inquire about other Waterborne, Inc. products, please call, FAX, or email us. Also, please visit our website at: www.waterborneinc.com.

Reference(s):

1. Slifko, T.R., et al. 1999. Applied Environmental Microbiology. 65: 3936-3941.

Waterborne, Inc., Clinical and Environmental Parasitology Products, 6045 Hurst Street, New Orleans, LA 70118 USA
Tel/Fax: 504-895-3338. Toll-free: 866-895-3338, Email: custserv@waterborneinc.com, Website: www.waterborneinc.com