A710FLR-1X. MicrosporUltra-FA™, FL, 1x conc., Reagent Only

FL-labeled Antibody Reagent for Direct Immunofluorescence Detection of *Encephalitozoon* spp. and *Enterocytozoon bienusi* Spores in Water Samples

Explanation: The A710FLR-1X kit is intended for use in immunofluorescence detection of spores of *Encephalitozoon* species and *Enterocytozoon beinusi* spores in environmental samples.

Description of Products

» This kit utilizes the principle of direct immunofluorescence. The reagent consists of two fluorescein (FL)-labeled monoclonal antibodies. One monoclonal antibody was made to outer spore wall antigenic sites (epitopes) of *Encephalitozoon intestinalis*; the other antibody was made to outer spore wall antigenic sites (epitopes) of *Enterocytozoon bienusi*. The reagent will bind to spores of *E. intestinalis, E. hellum,* and *E. cuniculi* and *E. beinusi* if they are present. The spores measure approximately 1 um by 2.5 um; however, it should be noted that the size and shape of spores can vary greatly (0.5-1.2 um by 1.5-2.5 um)[1,2]. The spores will appear bright apple-green when viewed under a fluorescence microscope using the appropriate filters for fluorescein.

» Reagent consists of 3.5 mL of a working-dilution (1X) solution of two fluorescein (FL)-labeled monoclonal IgG antibodies prepared against spores of *Encephalitozoon intestinalis* and *Enterocytozoon beinusi*. The volume provided is enough reagent for at least 75 tests using one drop per test on well slides, approximately 45 uL per drop, on slide wells. The antibody reagent contains 0.04% w/v sodium azide as preservative and 1% bovine serum albumen as antibody stabilizer. The antibody has been proven to react strongly with spores of *E. intestinalis, E. cuniculi, E. hellem,* and *E. beinusi*.

» There are two positive control suspensions for this product - the first consists of *E. intestinalis* spores (from in vitro culture) in a buffered aldehyde fixative. The concentration of this suspension is approximately 2x10e5 spores per mL. The second suspension is ~1.0 mL of *E. bienusi* positive fecal suspension in a buffered aldehyde fixative. The concentration of spores in this suspension has not been determined.

Storage: Store at 4° C. DO NOT FREEZE.
A710FLR-1X reagent is light sensitive.

Kit Includes:

- A710FLR-1X: 1 dropper vial containing 3.5 mL working dilution (1x) reagent
- 2 vials containing positive control suspensions

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.
Email: contact@waterborneinc.com

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. When the slide has dried completely, pass a small flame quickly under the wells of the slide 3 times. (One second per pass with the flame). This will "heat-fix" the spores if they are present.

3. Apply one drop (approximately 45 uL) of Micospor-FA™ 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.

4. 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.

5. 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.

6. 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.

7. 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.

8. 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.

2. 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.

3. 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. Ombruck et al. 1996. C. R. Acad. Sci. Paris, 319: 39-43.
2. Aldras et al. 1994. Journal of Clinical Microbiology. 32: 608-612.

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