



ST114K. MicrosporUltra-FA™ Comprehensive Kit

Fluorescein-labeled Antibody Reagent for Direct Immunofluorescence

Detection of Spores of *Encephalitozoon* spp. and *Enterocytozoon bienusi* in Stool Samples

Explanation: The ST114K kit is intended for use in immunofluorescence detection of spores of *Encephalitozoon* species and *Enterocytozoon bienusi* in stool samples.

Description of Products:

- » Reagent consists of 3.5 mL of a working-dilution (1X) solution of a fluorescein (FL)-labeled monoclonal IgG antibody prepared against spores of *Encephalitozoon intestinalis* and a fluorescein (FL)-labeled monoclonal IgG antibody prepared against spores of *Enterocytozoon bienusi*. The volume provided is enough reagent for at least 75 tests using one drop per test, 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*, and *E. hellem* and *E. bienusi*.
- » 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.
- » 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 consists of two vials, the first is a suspension of *E. intestinalis* spores (from in vitro culture) in a mixed aldehyde buffer and the second is 1 mL of fecal material containing spores of *E. bienusi*.

Storage: Store at 4° C. DO NOT FREEZE.
ST114R and M101 are light sensitive.

Kit Includes:

- ST114R: 1 dropper vial containing 3.5 mL working dilution (1x) reagent
- C101: 1 dropper vial containing 3.5 mL BlockOut™ counterstain
- M101: 1 dropper vial containing 3.5 mL No-Fade™ Mounting Medium
- PC101: 1 screw cap vial containing 1.0 mL positive control
- 1 microtube containing 1.0 mL positive control
- S100-2: 1 box of two-well SuperStick™ Slides, 40/box
- WB101: 1 screw cap bottle containing 50 mL 1x SureRinse™ Wash Buffer

Other Lab Supplies Not Included, but Available:

- B100-40: 40 mL Dilution/Blocking (D/B) Buffer
- D10 1: 0.4 mL DAPI, 5000X in methanol
- M102: 1 dropper vial containing 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
- S100-3: Three-well SuperStick™ Slides, 40/box
- WB100: 1 screw cap bottle containing 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.
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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.
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.
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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.

Other Information, Tips & Troubleshooting

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