**ACINF Staining kit for influenza A and B using the Immunofluorescence technique. 80 test.**

**KIT CONTENTS:**
- 1 ml of FITC-labelled monoclonal antibody specific for the influenza A nucleoprotein diluted in phosphate buffered saline containing bovine albumin, sodium azide and Evans blue as counterstain.
- 1 ml of FITC-labelled monoclonal antibody specific for the influenza B nucleoprotein diluted in phosphate buffered saline containing bovine albumin, sodium azide and Evans blue as counterstain.
- 1 positive (MDCK cells infected with influenza A ) and negative (non-infected MDCK cells ) control slides.
- 1 positive (MDCK cells infected with influenza B) and negative (non-infected MDCK cells) control slides.

**Materials required but not included in the kit:**
- Adequate precision micropipettes.
- Centrifuge with swinging rotor giving at least 1500 g.
- 37°C incubator.
- Distilled water.
- PBS (phosphate buffered saline pH 7.0-7.5).
- 24x60 coverslips.
- Fluorescence microscope and suitable filters according to the manufacturer’s recommendations.
- DPX.
- Mounting medium (buffered glycerin).
- Humid chamber for incubation of slides.

**CONSERVATION:**
Store at 2-8°C. Do not use the kit reagents beyond the expiration date printed on the label. Kits are stable through the expiration date when stored closed and at the temperature indicated. The cells used in the inoculation process should be store at 34-38°C.

**STABILITY AND HANDLING OF REAGENTS:**
Handle reagents in aseptic conditions to avoid microbial contaminations. Use only the amount of reagents required for the test. Do not return the excess solution into the bottles.

**VIRCELL SL does not accept responsibility for the mishandling of the reagents included in the kit.**

**RECOMMENDATIONS AND PRECAUTIONS:**
1. Use kit components only. Do not mix components from different kits or manufacturers.
2. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material. Use only sterile material for the inoculation process.
3. Do not use in the event of damage to the package.
4. Never pipette by mouth.
5. Inoculation medium, monoclonal and conjugates in this kit include substances of animal origin. Positive controls contain fixed antigens. Samples may the control and patient specimens be handled as potentially infectious. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
6. Monoclonal and conjugate contain sodium azide (concentration <0.1%). Avoid contact with acids and heavy metals.
7. Evan’s blue (concentration <0.1%) is a carcinogen. Avoid contact with skin or eyes. In case of contact with this solution, rinse thoroughly with water and seek medical attention.
8. Use only protocols described in this insert. Incubation times and temperatures other than specified may give erroneous results.
9. Microscope optics, light source condition and type will affect the fluorescence quality.
10. Do not leave the reagents at RT longer than absolutely necessary.

**PRELIMINARY PREPARATION OF THE REAGENTS:**

All reagents are ready to use.

**ASSAY PROCEDURE:**

**STAINING TECHNIQUE FOR CELL MONOLAYER.**
1. Disrupt the cell monolayer by means of a Pasteur pipette.
2. Wash twice with PBS by centrifugation at 1000 g.
3. Prepare smears with 25 µl of the pellets in a multiwell slide.
4. Air dry the slide and follow from step 4 of the procedure described below.

**STAINING SHELL-VIAL TECHNIQUE:**
1. Incubate the inoculated vials at 37°C until scheduled for use.
2. Dispose of the medium and the coverslip by punching the bottom of the vial with a red-hot needle. Pick the coverslip with forceps (be care not to damage the cell monolayer) and air dry it.
3. Adhere to a slide with DPX with the cell monolayer facing upwards (the cell-containing side appears opaque under light). Press slightly the coverslip against the slide with the help of a pipet tip to avoid bubbles.
4. Fix for 10 minutes with acetone.
5. Add 25 µl of FITC-labelled monoclonal antibody. Carefully spread over the whole monolayer and incubate for 30 minutes at 37°C in a humid chamber in the darkness.
6. Wash in PBS for 10 minutes.
7. Dry and add one drop of buffered glycerin. Place a coverslip and press slightly to avoid bubbles. View under the fluorescence microscope at 400x.

**STAINING TECHNIQUE FOR DIRECT DETECTION IN SAMPLES:**
The samples for direct detection may be nasopharyngeal swabs, pharyngeal swabs, bronchoalveolar lavage, bronchial wash and nasopharyngeal aspirate.

Swabs specimen should be placed into 1 to 2 ml of viral transport medium. Do not prepare slides from dried swabs.

Fluid specimen should be placed into a sterile container.

If the specimen is not tested immediately, store at 2°C to 8°C overnight or freeze at -80°C.

**CELL SPOT PREPARATION:**
A. Aspirates and fluids containing cells:
1. Transfer specimen to a conical centrifuge tube and centrifuge at 300 to 500 g for 10 minutes at 4°C.
2. Remove and discard the supernatant, including the mucus that may be overlaying the cell pellet in respiratory samples.
3. Tap the tube gently to loosen the cells, add PBS, and suspend the cells by gently pipetting up and down.
4. Repeat the centrifugation step.
5. Remove and discard the supernatant.
6. Tap the tube and add enough PBS to yield an opalescent cell suspension.
7. Deliver approximately 35 µl of the cell suspension in the well of a Teflon-coated slide or normal glass slides and see under the microscope to ensure that the cell concentration is adequate.
8. Air dry the slides completely at room temperature.
9. Fix the cells by immersing the slide in acetone for 10 minutes at 4°C.
10. Remove the slides from acetone and allow them to air dry completely at room temperature.
B. Swab specimens or scrapings:
1. These samples should be transported in viral transport medium. Vortex the tube containing the swab in order to release cells.
2. Follow the protocols as per point A described above.

Slide may be stored for 2 to 3 days at 4°C in an airtight container with desiccant or at ~20°C for longer storage. When removing the slide from storage, allow them to equilibrate at room temperature before opening the storage container.

STAINING PROCEDURE:
1. Add 25 µl of FITC-labelled monoclonal antibody to each well or slide and incubate for 30 minutes at 37°C in a humid chamber.
2. Wash with PBS for 10 minutes.
3. Dry and add buffered glycerin (pH> 8). Place a coverslip and press slightly to avoid bubbles.
4. View under the fluorescence microscope at 400 x.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal QC testing before release.

VALIDATION PROTOCOL FOR USERS:
Positive and negative controls should be included into each test run. It allows the validation of the assay and kit. The observed fluorescence pattern should be:
Positive control: characteristic apple-green fluorescence in the whole cell.
Negative control: no fluorescence.

INTERPRETATION OF RESULTS:
The reaction is positive when characteristic apple-green fluorescence in the whole cell can be observed. The reaction is negative when no fluorescence is observed.
A negative result does not exclude an infection by influenza A or influenza B.

LIMITATIONS:
1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
2. A negative result does not exclude an infection by influenza A or influenza B.

LITERATURE:

For any question please contact: customerservice@vircell.com

REVISED: 07/2008