MONOFLUOTM KIT

MONOFLUOTM KIT INFLUENZA 2 X 45 TESTS 52209
MONOFLUOTM KIT ADENOVIRUS 45 TESTS 52210
MONOFLUOTM KIT PARA-INFLUENZA 1+2 45 TESTS 52211
MONOFLUOTM KIT PARA-INFLUENZA 3 45 TESTS 52212

DETECTION OF INFLUENZA A AND B, PARAINFLUENZA 1 AND 2, PARAINFLUENZA 3 OR ADENOVIRUS BY IMMUNOFLUORESCENCE

IVD
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1- CLINICAL VALUE

The Respiratory Syncytial, Influenza A and B, para-Influenza 1, 2 and 3 viruses and Adenovirus are responsible for serious respiratory infections in infants and old or weakened people. The diagnosis of the viral or bacterial origin of these infections is essential to implement appropriate therapeutic measures.

The diagnosis of these viral diseases is based on the isolation of the virus. Indeed, the serological diagnosis does not yield satisfactory results; the antibody titers significantly increase only 10 to 15 days after the beginning of the clinical signs and cannot usually be detected in infants. The reference method remains the isolation of the virus from cell culture (Hela, KB, Hep2 cells or fibroblastic cell lines of embryo origin) and its identification, from a naso-pharyngeal specimen or broncho-alveolar lavage fluid. The specimen is obtained during the phase of maximum virus excretion, 1 to 6 days after the onset of the disease.

The association of the monoclonal antibodies specific to these viruses with respiratory tropism and the immunofluorescence technique allowed the rapid diagnosis of these diseases (by direct examination of the specimen) without resorting to the cell culture method, which is difficult to perform.

For each of these viruses, monoclonal antibodies directed against the virus proteins have been selected, first because of their specificity, and secondly because of the quality of the image observed during an immunofluorescence application; the usual appearance is a clearly visible granular or particulate fluorescence in the cytoplasm of infected cells. These monoclonal antibodies may also be used to identify each of these viruses after their isolation from cell culture.

The prevalence of these various infections leads Bio-Rad to propose the elaboration of the rapid diagnosis of diseases of viral etiology as follows:

- The Respiratory Syncytial Virus (RSV) is responsible for more than 60% of these infections; MONOFLUO™SCREEN R.S.V. is used to identify this virus by a direct immunofluorescence technique in one step on the collected specimen.
- The Influenza A and B, para-Influenza 1, 2 and 3 viruses and Adenovirus cause, in various ways according to the regions and the periods of the year, about 30% of these infections. MONOFLUO™SCREEN INFLUENZA, PARA-INFLUENZA, ADENOVIRUS is used to confirm or invalidate the presence of one of these viruses by a
direct immunofluorescence technique in one step on the specimen; in case of positive response, the incriminated virus can be identified using the:

- **MONOFLUO™ KIT INFLUENZA** (code 52209)
- **MONOFLUO™ KIT PARA-INFLUENZA 1 + 2** (code 52211)
- **MONOFLUO™ KIT PARA-INFLUENZA 3** (code 52212)
- **MONOFLUO™ KIT KIT ADENOVIRUS** (code 52210)

2- **PRINCIPLE**

The MONOFLUO™ KIT Influenza test kit is intended for the detection of the Influenza A and B virus in infected cells by means of indirect immunofluorescence technique using monoclonal antibodies specific to each of these viruses.

The MONOFLUO™ KIT Adenovirus test kit is intended for the detection of the different strains of Adenovirus.

The MONOFLUO™ KIT para-Influenza 1+2 test kit is intended for the detection of the types 1 or 2 para-Influenza virus, and MONOFLUO™ KIT para-Influenza 3 test kit for the detection of para-Influenza virus type 3. These monoclonal antibodies bind to the antigen expressed in the cytoplasm of infected cells obtained from specimens containing secretions or cell exudates from the respiratory tract, or after isolation of the virus from cell cultures.

The addition of mouse anti-IgG conjugate labeled with fluoresceine makes the cells which have fixed the monoclonal antibody fluorescent. The cells that bind to the specific monoclonal antibodies directed against the virus proteins exhibit fluorescence, mainly cytoplasmic, with a granular or particulate appearance at the fluorescent microscope examination.

3- **COMPOSITION OF THE KIT**

For storage conditions and expiration date refer to the box label. Reagents stored at +2-8°C, in absence of microbial contamination, are stable until the expiry date indicated on the label (even once opened).
**MONOFLUO™ KIT INFLUENZA (code 52209)**

<table>
<thead>
<tr>
<th>LABEL</th>
<th>REAGENTS</th>
<th>PRESENTATION</th>
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</thead>
<tbody>
<tr>
<td><strong>R1a</strong> Monoclonal</td>
<td>Monoclonal antibodies (mouse)</td>
<td>1 x 2,5 ml (dropper vial)</td>
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<tr>
<td>Antibody</td>
<td>Anti-Influenza A (clone IA52/9)</td>
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<td>anti-Influenza A</td>
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<td>Anti-Influenza B (clone IB82/2)</td>
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<td>anti-Influenza B</td>
<td>Preservative: &lt; 0.1% sodium azide</td>
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<td><strong>R2</strong> Negative</td>
<td>Negative control (culture supernatant)</td>
<td>1 x 2,5 ml (dropper vial)</td>
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<tr>
<td>control</td>
<td>Preservative: &lt; 0.1% sodium azide</td>
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<tr>
<td><strong>R3</strong> Concentrated</td>
<td>Concentrated Conjugate (10X):</td>
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<tr>
<td>Conjugate (10X)</td>
<td>Antibody (sheep), mouse anti-IgG buffer labeled with fluorescein</td>
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</tr>
<tr>
<td></td>
<td>isothiocyanate containig Evans blue</td>
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</tr>
<tr>
<td></td>
<td>Preservative: &lt; 0.2% sodium azide</td>
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<tr>
<td><strong>R4</strong> Mounting</td>
<td>Mounting medium (ready to use):</td>
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<td>medium</td>
<td>Buffered Glycerol for immunofluorescence</td>
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<td></td>
<td>Preservative: &lt; 0.1% sodium azide</td>
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**MONOFLUO™ KIT ADENOVIRUS (code 52210)**

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<td><strong>R1</strong> Monoclonal</td>
<td>Monoclonal antibodies (mouse)</td>
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<tr>
<td>Antibody</td>
<td>anti-Adenovirus (clone H60 and H72)</td>
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<tr>
<td>Adenovirus</td>
<td>Preservative: &lt; 0.1% sodium azide</td>
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</tr>
<tr>
<td><strong>R2</strong> Negative</td>
<td>Negative control (culture supernatant)</td>
<td>1 x 2,5 ml (dropper vial)</td>
</tr>
<tr>
<td>control</td>
<td>Preservative: &lt; 0.1% sodium azide</td>
<td></td>
</tr>
<tr>
<td><strong>R3</strong> Concentrated</td>
<td>Concentrated Conjugate (10X):</td>
<td>1 x 1 ml (dropper vial)</td>
</tr>
<tr>
<td>Conjugate (10X)</td>
<td>Antibody (sheep), mouse anti-IgG buffer labeled with fluorescein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>isothiocyanate containig Evans blue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preservative: &lt; 0.2% d’azoture de sodium</td>
<td></td>
</tr>
<tr>
<td><strong>R4</strong> Mounting</td>
<td>Mounting medium (ready to use):</td>
<td>1 x 3 ml (dropper vial)</td>
</tr>
<tr>
<td>medium</td>
<td>Buffered Glycerol for immunofluorescence</td>
<td></td>
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<tr>
<td></td>
<td>Preservative: &lt; 0.1% d’azoture de sodium</td>
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### MONOFLUO™ KIT PARAINFLUENZA 1+2 (code 52211)

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<tr>
<td>R1</td>
<td>Monoclonal Antibody parainfluenza 1+2</td>
<td>Monoclonal antibodies (mouse) anti-parainfluenza 1 et 2 (clone P2 128/14) Preservative: &lt;0.1% sodium azide</td>
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<tr>
<td>R2</td>
<td>Negative control</td>
<td>Negative control (culture supernatant) Preservative: &lt;0.1% sodium azide</td>
</tr>
<tr>
<td>R3</td>
<td>Concentrated Conjugate (10X)</td>
<td>Concentrated Conjugate (10X) : Antibody (sheep), mouse anti-IgG buffer labeled with fluorescein isothiocyanate containing Evans blue Preservative: &lt;0.2% d'azoture de sodium</td>
</tr>
<tr>
<td>R4</td>
<td>Mounting medium</td>
<td>Mounting medium (ready to use) : Buffered Glycerol for immunofluorescence Preservative: &lt;0.1% d’azoture de sodium</td>
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### MONOFLUO™ KIT PARAINFLUENZA 3 (code 52212)

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<tr>
<td>R1</td>
<td>Monoclonal Antibody parainfluenza 3</td>
<td>Monoclonal antibodies (mouse) anti-parainfluenza 3 (clone Pi3 5/12) Preservative: &lt;0.1% sodium azide</td>
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<tr>
<td>R2</td>
<td>Negative control</td>
<td>Negative control (culture supernatant) Preservative: &lt;0.1% sodium azide</td>
</tr>
<tr>
<td>R3</td>
<td>Concentrated Conjugate (10X)</td>
<td>Concentrated Conjugate (10X) : Antibody (sheep), mouse anti-IgG buffer labeled with fluorescein isothiocyanate containing Evans blue Preservative: &lt;0.2% d’azoture de sodium</td>
</tr>
<tr>
<td>R4</td>
<td>Mounting medium</td>
<td>Mounting medium (ready to use) : Buffered Glycerol for immunofluorescence Preservative: &lt;0.1% d’azoture de sodium</td>
</tr>
</tbody>
</table>
4- **PRECAUTIONS OF USE**

Quality of results is dependent upon the respect of the following good laboratory practices:

- Do not use expired reagents.
- Do not mix nor combine reagents from kits having different batch numbers during the same procedure.
- Before use, let the reagents stabilize at room temperature (+18-30°C).
- Use glassware thoroughly washed and rinsed with de-ionized water, or preferably, disposable material.
- Use a new pipette tip for each sample.
- Check the quality of the de-ionized water. If fluorescent organisms are observed on a negative control, it is necessary to sterilize the water used by filtration.
- Do not allow the conjugate to dry on the slide during staining.

**HEALTH AND SAFETY INSTRUCTIONS**

- Wear disposable gloves.
- Do not "pipette by mouth".
- Consider any material directly in contact with specimens as contaminated, hence infectious materials.
- Avoid spilling.
- Always comply with current techniques and precautions concerning protection against microbiological hazards, for handling and elimination of material and biological products used for the reaction.

**CAUTION:** R3 reagent (concentrated conjugate) contains sodium azide at a concentration < 0.2%.

R22-32 : Harmful if swallowed. Contact with acids liberates very toxic gas.

S28-60 : After contact with skin, wash immediately with plenty of water. This material and its container must be disposed of as hazardous waste.

Material safety data sheet is available upon request.
5- SPECIMEN

These viruses are confined to secretions and cell exudates from the upper respiratory tract.
To perform direct diagnosis by means of fluorescence, collect desquamated cells from nasal or tracheo-bronchial secretions. If the virus is to be isolated from cell cultures, the immunofluorescence technique may also be applied to cells which have been infected in vitro, as soon as the cytopathic effect is detectable.

5.1. TECHNIQUE FOR SPECIMEN COLLECTION

The Influenza A and B, para-Influenza 1, 2 and 3 viruses and Adenovirus are investigated in specimens containing nasal or tracheobronchial secretions obtained by swabbing with a cotton Q-tip® or by aspiration or nasal lavage with PBS buffer solution. For direct examination of specimens in immunofluorescence, the respiratory secretions may be transferred into the laboratory.

In case of viral isolation, the secretions are collected in an appropriate transport medium to maintain the virus: preferably, saline solution – or buffered MEM medium (since some viruses with respiratory tropism lose their infectious nature in an acid medium) and enriched with certain substances to protect the viruses: bovine albumin, gelatine, chicken serum, saccharose and antibiotics. The following formula is an example:
• MEM, 5 mg/ml bovine albumin, 4.76 mg/ml HEPES, 0.22 mg/ml sodium bicarbonate, 1 500 U/ml penicillin, 1 000 mcg/ml streptomycin.

For isolation, the specimen should be transported frozen or cold. A key element is to obtain a sufficient amount of secretions. It is usually more effective and convenient to collect respiratory secretions, very often mucous, by aspiration, using a small tube connected to a specimen collection bottle, with the tube inserted into the child's nose. This system is commercially available under the name "mucosity suction pump". When aspiration cannot be performed at the child's bedside, a large capacity (50 ml) syringe, a manual vacuum pump, or, if possible, an electrical aspiration system may be used.

Sometimes, for example in bronchiolitis cases, the child's nose is dry and it is not possible to obtain a sufficient quantity of secretion. In this case, lavage with a nasal enema may be used: a few millimeters of solution are instilled into one nasal passage and then this fluid is immediately aspirated.
In most cases, nasal aspiration allows the collection of, at least, 0.2 to 0.5 ml of secretions. This material is then transferred into the laboratory, or placed in the appropriate transport medium for virus isolation on cell culture.

At room temperature (18-30°C), storage for less than 3 hours will not alter the quality of the viral antigens in the specimen. Cold storage (+2 - 8°C) is recommended for a viral isolation, but the inoculation of cell cultures must not be delayed too long since its infectious power decreases even after deep freezing at -70°C.

5.2. TREATMENT OF SPECIMENS BEFORE I.F. EXAMINATION

In the laboratory, several successive washings of the aspirated material are necessary to obtain a suspension of mucous-free nasal cells.

• Prepare necessary volume of PBS buffer for treatments and washings by diluting concentrated solution 10 times in sterile distilled water.
• Add 5 ml of PBS to approximately 0.5 - 1 ml of secretions. Gently shake.
• Centrifuge for 10 minutes at 500 g at +2 - 8°C. Decant the supernatant fluid.
• Add 5 ml of PBS to the precipitate and centrifuge. Repeat the washing procedure 2 or 3 times to completely eliminate any mucous.
• After the final centrifugation, add 1 ml of PBS to the cell precipitate. Homogenize the suspension by pipetting.
• Apply the cells onto the slides.
• Perform cell fixation and staining (see staining technique).

5.3. VIRUS ISOLATION ON CELL CULTURE

Since certain of these viruses with respiratory tropism are thermolabile, it is important to inoculate cell cultures as soon as possible after specimen collection.

In the laboratory, freeze and thaw test specimens so that the cells burst open and release virus particles. Centrifuge for 10 minutes at 500 g at +2 - 8°C to remove cellular debris (2,000 rpm).

Inoculate the cell culture intended for this purpose with the specimen obtained. The cells used are Hep2, Hela or KB, as well as diploid cell lines from human embryonic fibroblasts in a MEM medium containing 5 to 8 % of fetal calf serum.
Depending on the bottle used for cell culture, the volumes of inoculum and of culture medium will differ.

- If Leighton tubes are used, inoculate the culture with 0.3 ml of specimen. Allow to remain in contact for 2h30 at 37°C then dilute to 1.2 ml with the culture medium.
- If a 25 cm³ bottle is used, inoculate with 1 ml of specimen. Incubate at +37°C for 2h30 then dilute to 12 ml with the culture medium. Incubate the cells at +37°C until the cytopathic effect of the virus is observed (about 4 to 6 days).

**Observation of the cytopathic effect**

Observe inoculated cell with inverse phase optical microscope and follow development of cytopathic effect: progressive separation of more or less rounded and refringent small cells with some nuclear inclusions for the Influenza and para-Influenza viruses, large rounded cells forming aggregates for Adenovirus.

If, after 6 to 8 days, the cytopathic effect is still not observed, after inoculating a specimen, proceed with staining anyway. Viral multiplication could be sufficient to be detected at this stage by immunofluorescence.

A second passage onto cells may then be envisaged.

**6- ASSAY PROCEDURE**

**6.1 - MATERIAL REQUIRED BUT NOT PROVIDED**

- Distilled or fully de-ionized water
- Bleach
- Absorbent paper
- Disposable gloves
- Phosphate buffer (PBS) pH 7.2 for IF (concentrated 10X) - 50 ml - code 74901
- Acetone
- Sterile Pasteur pipettes
- Automatic or semi-automatic, adjustable or preset, pipettes to dispense 10 to 200µl and 1mL.
- Graduated test tubes of 10 ml or 25 ml capacity
- Disposable tubes
- Tubes for centrifugation
• Immunofluorescence slides - code 50569 (2 wells) or 50566 (6 wells)
• Cover slips
• Fluorescent microscope
• Biohazardous waste container

6.2 - RECONSTITUTION AND STORAGE OF REAGENTS

R3: Dilute vial content with 9 ml phosphate buffer to obtain ready-to-use solution dilution. After dilution, mouse anti-IgG conjugate, stored at +2-8°C and in the absence of microbial contamination, is stable for 3 months.

6.3 - PROCEDURE

6.3.1. CELL APPLICATION AND FIXATION

• Direct examination on specimen
  - Dispense 20 µl of the treated specimen (cell centrifugation residue) into a slide well.
  - Air dry the slides using a drier or at room temperature (18-30°C).
  - Fix in an acetone bath at -20°C for 5 minutes.
  - Allow the slides to air dry.

• Cell culture on slide
  - Wash the slide twice for 1 minute in a PBS bath.
  - Allow the slide to air dry.
  - Fix in an acetone bath at -20°C for 5 minutes.

• Cell culture in bottles
  - Remove the medium. Collect the cells by scraping in 1 ml of PBS.
  - Resuspend the cells by aspirating and discharging several times, using a tapered pipette.
  - Then proceed for cell application and fixation as for direct examination on specimen.
6.3.2. ANTIBODY APPLICATION

1. Prepare the required volume of phosphate buffer (PBS) for washing by diluting the 10 times concentrated solution in sterile distilled water.
2. Prepare Conjugate R3 (refer to chapter 6.2)
3. Dispense a drop of specific monoclonal antibodies R1 (R1a and R1b for Influenza A and B) into the first wells (first and second wells for Influenza A and B) making sure that the whole circle surface is covered.
4. Dispense a drop of negative control (R2) into the following well.
5. Incubate the slide for 30 minutes at +37°C in a humid incubator.
6. Once the incubation time is over, gently wash the slide with PBS using a wash bottle.
7. Wash twice for 2 to 5 minutes with PBS. Gently agitate.
8. Allow the slide to air dry.
9. Shake gently the diluted conjugate (R3); dispense a drop of diluted conjugated (R3) on each well.
10. Incubate for 30 minutes at 37°C in a moist chamber.
11. After incubation, gently wash the slide with PBS using a wash bottle.
12. Wash twice for 2 to 5 minutes with PBS. Gently agitate.
13. Dip the slide (a few seconds) into distilled water.
14. Allow the slide to air dry.
15. Mount with cover slip using the buffered glycerol (R4). Check that the slide is free of air bubbles. Lute the slide with the varnish.

7- INTERPRETATION OF THE RESULTS

7.1 - QUALITY CONTROL
Stain at the same time a reference slide using positive and negative cells.

7.2 – READING AND INTERPRETATION OF THE RESULTS
Examine the slides using a fluorescent microscope at X100 and X400 magnification:
• Reading of negative control well: no fluorescent cell is observed after examination of the entire well.
• Positive reaction: intra-cytoplasmic granular fluorescence among other redish cells; at least one characteristic fluorescent cell is observed.
• Negative reaction: no fluorescent cell is observed after examination of all the wells.

*Note: Read the slides immediately to obtain better results. However the slides can be stored at +2 - 8°C in the dark for 24 hours.*

### 8- Limits of the Test
Diagnosis of recent infection can only be established on the basis of a combination of clinical observations and serological data. The result of a single test does not constitute sufficient proof for diagnosis of recent infection.

### 9- Quality Control of the Test
MONOFLUO™ KITS performances are controlled using slides coated with positive and negative controls. They are tested with monoclonal antibodies specific of each virus and with negative supernatant. The results are the following:

- with monoclonal antibodies: presence of fluorescent cells, intensity ≥ ++
- with culture supernatant: no fluorescent cells.

### 10- Quality Control of the Manufacturer
All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

### 11- References


8. LUCAS G., POTHIER P., DELAGNEAU J.F. Diagnostic rapide du Virus Respiratoire Syncytial (VRS) par immunofluorescence indirecte à l'aide d'anticorps monoclonaux. Biologie Prospective - 6ème colloque, 1985, Pont à Mousson, France.


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(US) - The other languages which are required in conformity to the European Directive can be obtained from your local Bio-Rad agent.

(FR) - Les autres langues requises par la Directive Européenne sont disponibles auprès de votre représentant Bio-Rad local.

(ES) - Los otros idiomas que se requieren para la conformidad de la Directiva Europea pueden ser obtenidos en su oficina local Bio-Rad.

(IT) - Le altre lingue che sono richieste in conformità con le Direttive Europee possono essere ottenute dal locale agente Bio-Rad.

(DE) - Die anderen Sprachen, die in Übereinstimmung mit der europäischen IVD Direktive benötigt werden, erhalten Sie über Ihre lokale Bio-Rad Niederlassung.

(PT) - Óvriga språk som krävs i enlighet med EG-direktivet kan erhållas från din lokala Bio-Rad-representant.

(DK) - De øvrige sprog som kræves i henhold til EU direktiv kan fås ved henvendelse til den lokale Bio-Rad leverandør.

(GR) - Τις υπολογίσεις χρήσεις που απαιτούνται για συμμόρφωση στην ευρωπαϊκή οδηγία μπορείτε να τις προμηθεύεστε από τον τοπικό υπολόγισης Bio-Rad.

(PL) - Tłumaczenie w innych językach które są wymagane w Dyrektywie Unijnej może być otrzymane od lokalnego przedstawiciela firmy Bio-Rad.

(LT) - Vertimasis, reikalavimas pagal Europos saugumo direktyvos reikalavimus, j kitas kalbas galite gauti iš vietinio Bio-Rad atstovo.

(H) - A leírás az Európai Irányelv által előírt egyéb nyelvenek hozzáférhető a Bio-Rad helyi kirendeltségeinél.

(EST) - Teised vastavalt Euroopa Direktiivile nõutavad keeled on saadaval kohaliku Bio-Rad edasimüüja kliest.

(SK) - Ostatné jazykové verzie, ktoré sú vyžadované v zhode s Európskou direktívou, možno obdržať od vášho lokálneho zástupcu Bio-Rad.

(CZ) - Další jazykové verze vyžadované ve shodě s evropskou direktivou jsou k dispozici u lokálního zastoupení firmy Bio-Rad.