RUO

CYTOMEGALOVIRUS MAb

For research use only

ACCMV: Kit for detection of cytomegalovirus using the immunofluorescence technique. 80 test.

INTRODUCTION:

Cytomegalovirus (CMV) is a member of the herpesvirus family and characteristically produces latent infection after primo infection. It is an important agent of congenital infection and can produce life-threatening illness in transplant and AIDS patients. An active infection resulting from a primary or reactivated latent infection during pregnancy may be transmitted to the fetus or to the infant during birth. The children congenitally infected can have severe central nervous system damage. A primary infection in adults may be asymptomatic or result in various syndromes, including mononucleosis, hepatitis or pneumonitis. The presence of active CMV infection can be detected by serological methods, but have to be confirmed by using viral isolation, antigen or nucleic acids detection.

Virus isolation is carried out in cell culture. Early pre-cytopathic effect detection of infected cultures can be performed by staining of centrifugation-enhanced shell vials with a monoclonal antibody against immediate early antigen at 24 hours after specimen inoculation.

PRINCIPLE OF THE TEST:

The Inmunofluorescence method is based upon the reaction of specific antibodies contained in the kit with the antigen in the sample. The antibodies react with the antigen, and the not binded are removed in the washing step. The antigen-antibody complexes are visualized with the aid of the fluorescein conjugated to the antibody directly or by means of a secundary antibody.

KIT FEATURES:

All reagents, except for the VIRCELL PBS 2, are supplied ready to use. All the reagents have a number assigned for an easy identification. In the Assay Procedure, the numbers of the reagents to be used in each step are indicated.

KIT CONTENTS:

1 VIRCELL CMV MAb: 2 ml of monoclonal antibody specific for CMV immediate early antigen (72 kD) diluted in phosphate buffered saline containing bovine albumin and sodium azide.
2 VIRCELL PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.
3 VIRCELL CMV CONTROL: 2 positive (MRC-5 cells infected with AD-169 cytomegalovirus strain) and negative (non-infected MRC-5 cells) control slides.
4 VIRCELL FITC-Ab: 2 ml of FITC-labelled anti-mouse IgG diluted in phosphate buffered saline containing bovine albumin, sodium azide and Evans blue as counterstain.
6 VIRCELL MOUNTING MEDIUM: 3 ml of mounting medium: buffered glycerol, containing sodium azide.

Materials required but not included in the kit:

-Adequate precision micropipettes.
-Centrifuge with swinging rotor giving at least 1500 g.
-Thermostatized incubator.
-Distilled water.
-24x60 coverslips.
-Fluorescence microscope and suitable filters according to the manufacturer ´s recommendations.
-DPX
-Humid chamber for incubation of slides.

CONSERVATION:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. Kits are stable through the expiration date when stored closed and at the temperature indicated.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability and storage
Reconstituted VIRCELL PBS	4 months at 2-8ºC, never beyond its expiration date
Rest of the components	Refer to package label for expiration date (at 2-8ºC)

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. For research use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the VIRCELL PBS, VIRCELL FITC-Ab and VIRCELL MOUNTING MEDIUM solutions are compatible with the equivalents from other VIRCELL MAb references and lots. The rest of the components are compatible with other kits when the lot is the same.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. The VIRCELL MAb and VIRCELL FITC-Ab in this kit include substances of animal origin. VIRCELL CONTROL contain fixed antigens. Samples, VIRCELL CONTROL and patient specimens should 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.
7. VIRCELL MAb, VIRCELL FITC-Ab and VIRCELL MOUNTING MEDIUM contain sodium azide (concentration <0.1%). Avoid contact with acids and heavy metals.
8. VIRCELL MOUNTING MEDIUM contains glycerol. Avoid contact with acids and keep away from high temperatures.
9. 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.
10. Use only protocols described in this insert. Incubation times and temperatures other than specified may give erroneous results.

11. Microscope optics, light source condition and type will affect the fluorescence quality.
12. Do not leave the reagents at room temperature longer than absolutely necessary.
13. The glass elements contained in kits could cause physical damage in the event of break. Handle with care.

PRELIMINARY PREPARATION OF THE REAGENTS:
Only the VIRCELL PBS 2 must be prepared in advance. Add the contents of the vial 2 to 1 litre of distilled water. Shake it until the complete disolution. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:
STAINING SHELL-VIAL TECHNIQUE:
1. Dispose of the medium and fix for 10 minutes with methanol.
2. Remove 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. Add 25 µl of VIRCELL MAb 1. Carefully spread over the whole monolayer.
5. Incubate for 30 minutes at 36-38ºC in a humid chamber.
6. Rinse slide briefly with a gentle stream of PBS (avoid directing PBS at wells) and immerse for ten minutes in PBS. Dip wash slide briefly in distilled water and dry.
7. Add 25 µl of VIRCELL FITC-Ab 4. Carefully spread over the whole monolayer.
8. Incubate for 30 minutes at 36-38ºC in a humid chamber in the darkness.
9. Rinse slide briefly with a gentle stream of PBS (avoid directing PBS at wells) and immerse for ten minutes in PBS. Dip wash slide briefly in distilled water.
10. Dry and add one drop of VIRCELL MOUNTING MEDIUM 6.
11. Place a coverslip and press slightly to avoid bubbles.
12. View under the fluorescence microscope at 400x.

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

VALIDATION PROTOCOL FOR USERS:
Positive and negative controls should be included to test the correct performance each time a new kit opened. It allows the validation of the assay and kit.
The observed fluorescence pattern should be:
Positive control: characteristic apple-green fluorescent intranuclear inclusions.
Negative control: absence of fluorescence.

INTERPRETATION OF RESULTS:
DETECTION CELL CULTURE:
The reaction is positive when characteristic apple-green fluorescent intranuclear inclusions can be observed.
The reaction is negative when no fluorescence is observed.
The positive control is the best tool to determine the characteristics of the staining.

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 the tested virus.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures.
4. This kit has been validated for detection and identification of human CMV in cell culture.

PERFORMANCES:
- **SENSITIVITY AND SPECIFICITY:**

53 samples were assayed with CYTOMEGALOVIRUS MAb against another commercial available kit.
The results were as follows:

Sample nr	Sensitivity	Specificity
53	95%	100%

- **INTRA-ASSAY PRECISION:**

3 samples (2 positive and 1 negative) were individually assayed in groups of 5 in a single assay performed by the same operator in essentially unchanged conditions.
The same results were observed in all the assays.

- **INTER-ASSAY PRECISION:**

3 samples (2 positive and 1 negative) were individually assayed on 5 different conditions in which the operator or the test day were different.
The same results were observed in all the assays.

- **CROSS REACTIVITY AND INTERFERENCES:**

Samples known to be positive for other herpesvirus (enterovirus and Herpes simplex 1+2) and of the syndromic group (*Toxoplasma gondii*) were assayed.

The negative results of the test demonstrate the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

RUO	For research use only
	Use by (expiration date)
	Store at x-yºC
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

LITERATURE:

1. Arens, M., J. Owen, C. M. Hagerty, C. A. Reed, and G. A. Storch. 1991. Optimizing recovery of cytomegalovirus in the shell vial culture procedure. Diagn Microbiol Infect Dis 14:125-30.

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4. Paya, C. V., T. F. Smith, J. Ludwig, and P. E. Hermans. 1989. Rapid shell vial culture and tissue histology compared with serology for the rapid diagnosis of cytomegalovirus infection in liver transplantation. Mayo Clin Proc 64:670-5.

5. Reina, J., V. Fernandez-Baca, J. Saurina, I. Blanco, and M. Munar. 1998. Shell-vial culture and pp65 antigenemia assay in the detection of cytomegalovirus in the first blood sample of renal transplant recipients. J Med Virol 55:240-2.

6. Reina, J., J. Saurina, V. Fernandez-Baca, I. Blanco, and M. Munar. 1998. An increase in the number of polymorphonuclear leukocytes inoculated on shell-vial culture increases the sensitivity of this assay in the detection of cytomegalovirus in the blood of immunocompromised patients. Diagn Microbiol Infect Dis 31:425-8.

7. van der Bij, W., J. Schirm, R. Torensma, W. J. van Son, A. M. Tegzess, and T. H. The. 1988. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. J Clin Microbiol 26:2531-5.

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