ACCUFI-FA

CMV IMMUNOFLUORESCENCE ASSAY

CYTOMEGALOVIRUS PRE-CPE CULTURE IDENTIFICATION TEST

For Research Use Only
Catalog Number D-10001

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Intended Use

The BluePoint Bioscience Accufi-FA, Cytomegalovirus (CMV) Immunofluorescence Assay (IFA) is intended for use in pre-CPE culture confirmation with standard culture tubes and shell vials in the qualitative detection and identification of immediate early antigen of Human CMV.

This product is for research use only and is **not** intended for diagnostic use.

Summary and Explanation

Human cytomegalovirus is a member of the family *Herpesviridae*, and is in the betaherpesvirus subfamily. CMV is an enveloped, double-stranded DNA virus. Virions have a diameter of 200 – 300nm and contain an icosahedral nucleocapsid embedded in an amorphous tegument (matrix) surrounded by a phospho-lipid rich envelope. Viral replication occurs in the nucleus of the host cell after delivery of the viral genome and involves the expression of immediate-early, early and late classes of genes. The immediate early proteins have a significant effect on host cell and play a critical regulatory role in infection.

Test Principle

The Accufi-FA, CMV Immunofluorescence Assay is an indirect immunofluorescence test to qualitatively detect CMV immediate early antigen in infected cell cultures. Monoclonal antibodies, in the primary reagent, will bind to CMV immediate early antigen present in fixed cell preparations. Unbound primary antibody is removed by rinsing with wash buffer. DyLight™488 Anti-Mouse conjugate, in the secondary reagent, will bind to the monoclonal antibody-CMV antigen complex and unbound secondary antibody is removed by rinsing with wash buffer. The reacted fixed cell preparation is mounted for examination by fluorescence microscopy. A positive result is indicated by bright green fluorescence localized in the nucleus of infected cells. Non-infected cells will stain dull red from the Evans Blue counterstain.

Materials Provided

MAb CMV IE, Catalog No. C-10001. One dropper bottle containing a 5ml blend of monoclonal antibodies specific to CMV IE antigen, protein stabilizer, and preservative.

DyLight™488 Anti-Mouse, Catalog No. C-10002. One dropper bottle containing 5ml of DyLight™488 Anti-Mouse conjugate, Evans Blue, protein stabilizer, and preservative.

Mounting Fluid, Catalog No. C-10003. One dropper bottle containing 5ml of buffered glycerol, a fluorescence enhancer and preservative.

Materials Required But Not Provided

Masked microscope slide or coverslip with acetone/methanol fixed cell preparation
Humid chamber
Waterbath or incubator capable of maintaining 37°C (± 2°C)
Phosphate Buffered Saline/0.05% Tween 20 Wash buffer
Microscope slide (for mounting shell-vial coverslips)
Coverslips (for mounting microscope slides)
Fluorescence microscope equipped with optical filters optimized for DyLight™488 (excitation maximum 493nm, emission maximum 518nm)

Storage and Handling

Kits may be stored at 2-8°C. DO NOT FREEZE. Kit reagents should be at room temperature (15-30°C) when used for testing.

Warnings and Precautions

For research use only

Do not use kit components beyond the expiration date
Do not mix reagents from different lot numbers
Do not allow microscope slides or coverslips to dry at any time during the staining procedure
DyLight™488 Anti-Mouse reagent contains Evans Blue which is a potential carcinogen. If there is contact with the skin, wash immediately with running water.
The Mounting Fluid contains a fluorescence enhancer that may be destructive to mucous membranes. Avoid direct skin or mucous membrane contact. If there is contact with the skin or mucous membranes, wash immediately with running water.
Dispose of microscope slides in appropriate sharps disposal containers.

Specimen Collection and Preparation

This kit is intended for research use only, staining fixed cell preparations. For specimen collection and processing procedures refer to appropriate guidance manuals or protocols.

Staining Procedure

All kit components are provided ready-to-use.

1. Allow the kit components and fixed cell preparations to equilibrate to room temperature.
2. Add sufficient MAb CMV IE to completely cover the fixed cells of the microscope slide or shell-vial coverslip; 1 partial drop (10-20µl) for cell spots and 4-6 drops (160-200µl) for shell vial coverslips.
   Note: *dropper bottle tips deliver ~40µl/drop*
3. Incubate slides or shell-vials at 37°C for 30 minutes in a waterbath or incubator. Place microscope slides in a humid chamber prior to incubation to avoid reagent evaporation.
   Note: *do not allow the fixed cell preparations to dry at any time during the staining procedure.*
4. Shake or pour off excess reagent and thoroughly rinse slides or shell vials 3X with wash buffer. Slides and shell-vials may be rinsed using a squirt bottle. Rinse slides by directing the wash stream onto the mask next to the fixed cell spots then blot dry the mask surrounding the wells with a cotton swab. Rinse shell-vials by directing the wash stream onto the side of the vial adding enough wash buffer to completely submerge the cells fixed on the coverslip.
5. Add sufficient DyLight™488 Anti-Mouse to completely cover the fixed cells of the microscope slide or shell-vial coverslip; 1 partial drop (10-20µl) for cell spots and 4-6 drops (160-200µl) for shell vial coverslips.
6. Incubate slides or shell-vials at 37°C for 30 minutes in a waterbath or incubator. Place microscope slides in a humid chamber prior to incubation to avoid reagent evaporation.
7. Repeat wash as indicated in step 4.
8. Mount the slides or coverslips using the Mounting Fluid. Mount slides by adding 1 partial drop in each well and positioning the coverglass to reduce trapping air. Mount coverslips by adding 1 partial drop onto a microscope slide and positioning the coverslip **cell side down**. Avoid trapping air.
   Note: *coverslips may be dipped in distilled water just prior to mounting to eliminate "salt frost" during microscopic evaluation.*
9. Blot the edges of the mounted coverslips with a cotton swab or lab tissue to remove excess mounting fluid.
10. Examine slides using a fluorescence microscope with appropriate optical filters. Magnification of 200X should be sufficient to clearly identify fluorescence staining.

Interpretation of Results

A CMV positive control fixed cell preparation should be stained with test samples to ensure appropriate reagent activity and staining morphology.
A positive reaction is indicated by bright green fluorescence localized in the nucleus of CMV infected cells. Non-infected cells should exhibit no significant fluorescence and stain dull red due to the Evans Blue counterstain.

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