

ELISA kits available from ADI (see details at the web site)

Catalog#	ProdDescription
4200	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA kit
4205	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgM ELISA kit
4220-AHB	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) ELISA kit, Quantitative
4300-AHG	Human Anti-Hepatitis A Virus IgG (HAV-IgG) ELISA kit, Quantitative
4600	Human Anti-Hepatitis C Virus (Anti-HCV) ELISA kit, Semi-Quantitative
510-100-HRG	Human Anti-Rubella Virus IgG ELISA kit
510-110-HRM	Human Anti-Rubella Virus IgM ELISA kit
520-100-HMG	Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
520-110-HMM	Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative
520-120-HMA	Human Anti-Mumps Virus (parotitis) IgA ELISA, 96 tests, Quantitative
520-200-HVG	Human Anti-Varicella Zoster Virus (chickenpox) IgG ELISA, 96 tests, Quantitative
520-210-HVM	Human Anti-Varicella Zoster Virus (chickenpox) IgM ELISA, 96 tests, Quantitative
520-220-HVG	Human Anti-Varicella Zoster Virus (chickenpox) IgA ELISA, 96 tests, Quantitative
530-100-HMG	Human Anti-Measles IgG ELISA kit, 96 tests
530-110-HMM	Human Anti-Measles IgM ELISA kit, 96 tests
530-120-HMA	Human Anti-Measles IgA ELISA kit, 96 tests
540-100-DHG	Human Anti-Dengue Virus IgG ELISA kits, 96 tests, Quantitative
540-110-DHM	Human Anti-Dengue Virus IgM ELISA kits, 96 tests
600-020-HRV	Human Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative
600-120-HRV	Human Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 2x 96 tests,
600-220-HRV	Human Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 2x 96 tests,
600-300-100	Human Anti-Meningococcal Group A Oligosaccharides-Diphtheria CRM197 IgG
600-300-105	Human Anti-Meningococcal Group CWY Oligosaccharides-Diphtheria CRM197
600-300-115	Human Anti-Meningococcal Group ACWY Oligosaccharides-Diphtheria CRM197
600-370-CFP	Human Cardiac Fatty acid binding protein (FABP) ELISA kit
600-410-CTN	Human Cardiac Troponin-I (Tn-I) ELISA Kit
600-610-HMY	Human Myoglobin ELISA Kit
700-140-KLM	Human Anti-KLH IgG (total) ELISA Kit, 2x 96 tests, Quantitative
700-160-VAH	Human Anti-Vacumune/Immucothel (KLH) IgG (total) ELISA Kit, 2x 96 tests,
710-140-BSM	Human Anti-BSA IgG (total) ELISA Kit, 2x 96 tests, Quantitative
80170	Human Serum Antibody detection ELISA kit, Qualitative
900-160-83T	Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
910-160-JEM	Human Anti-Japanese encephalitis virus (JEV) IgG specific ELISA kit
910-170-JEM	Human Anti-Japanese encephalitis virus (JEV) IgM specific ELISA kit
920-040-HAG	Human Anti-Influenza A virus IgG ELISA kit
920-050-HAM	Human Anti-Influenza A virus IgM ELISA kit
920-060-HAA	Human Anti-Influenza A virus IgA ELISA kit
920-400-HBG	Human Anti-Influenza B virus Ig's ELISA kit
930-100-TTH	Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-100-DHG	Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-110-DHM	Human Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-200-DHG	Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit
940-210-DHM	Human Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit
950-100-AHA	Human Anti-Adenovirus IgA ELISA kit
950-110-AHG	Human Anti-Adenovirus IgG ELISA kit
950-120-AHM	Human Anti-Adenovirus IgM ELISA kit
960-200-PHA	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,
960-220-PHM	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,
960-250-PHG	Human Anti-B. pertussis Pertactin IgG ELISA kit
970-100-PHG	Human Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests
980-100-PHG	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit, 96
980-110-PHM	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgM ELISA Kit, 96
990-100-THA	Human Anti-Mycobacterium Tuberculosis IgA ELISA kit, 96 tests
990-110-THG	Human Anti-Mycobacterium Tuberculosis IgG ELISA kit, 96 tests
990-120-THM	Human Anti-Mycobacterium Tuberculosis IgM ELISA kit, 96 tests
AE-320420-1 tests	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgG ELISA Kit, 96
AE-320430-1 tests	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgM ELISA Kit, 96
AE-320520-1	Human Anti-Zaire-Ebola virus IgG ELISA Kit, 96 tests

Instruction Manual No. M-3300-375-CMM

Human Cytomegalovirus (CMV) IgM ELISA KIT

Cat. # 3300-375-CMM

For the detection of IgM antibody to Cytomegalovirus (CMV)
in human serum or plasma

For In Vitro Research Use Only (RUO)



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**DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE
KIT FOR ANY LOT SPECIFIC CHANGES.**

Kit Components (96 tests)	
CMV antigen coated strip plate, (8x12 strip or 96 wells) # 3300-375-P	1 plate
CMV IgM Calibrator, 1 ml # 3300-375-1, yellow cap	1 vial
CMV IgM Positive Control, 1 ml # 3300-375-2, red cap	1 vial
CMV IgM Negative Control, 1 ml 3300-375-3, blue cap	1 vial
Anti-Human IgM-HRP Conjugate, (12 ml) # 3300-375-4	1 bottle
Sample Diluent, 22 ml # 3300-375-5	1 bottle
Wash buffer (20X) 25 ml # 3300-375-WB	1 bottle
TMB Substrate Solution, 12 ml # 3300-375-TMB	1 bottle
Stop Solution, 12 ml # 3300-375-ST	1 bottle
Complete Instruction Manual	M-3300-375-CMM

Intended Use

ADI Cytomegalovirus (CMV) IgM ELISA Kit is intended for the detection of IgM antibody to CMV virus in human serum or plasma of normal or vaccinated individuals. This kit is for in vitro research use only (RUO).

Introduction

Human Cytomegalovirus (HCMV or CMV) is a member of the herpes group of viruses. It is also known as human herpesvirus-5 (HHV-5).[1] Within Herpesviridae, HCMV belongs to the Betaherpesvirinae subfamily, which also includes cytomegaloviruses from other mammals. Although they may be found throughout the body, HCMV infections are frequently associated with the salivary glands. After infection, HCMV remains latent within the body throughout life and can be reactivated at any time. Eventually, it may cause mucoepidermoid carcinoma and possibly other malignancies such as prostate cancer.

Most adults and children who catch CMV have no symptoms and are not harmed by the virus. CMV infection is of clinical significance primarily in pregnant women, newborn infants with possible congenital infection, immunosuppressed transplant patients and individuals with AIDS. Seroprevalence is age-dependent: 58.9% of individuals aged 6 and older are infected with CMV while 90.8% of individuals aged 80 and older are positive for HCMV. Significant increases in CMV IgG antibody by ELISA suggest recent infection or reactivation of a latent CMV infection. ELISA can detect CMV IgM antibody in both primary CMV infections (93-100%) and in reactivated infection (40%). An IgM response may be reduced or absent in immunocompromised patients with active infection. In transplant patients the CMV infection can be associated with higher morbidity and mortality.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2

INTERPRETATION

The following is intended as a guide to interpretation of CMV IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9 No detectable IgM antibody to CMV IgM.

0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.

>1.1 Detectable IgM antibody to CMV IgM.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.

2. Lipemic or hemolyzed samples may cause erroneous results.

Cross Reactivity

No cross reactivity to Corynebacterium diphtheriae.

References: Mangano WE (1996) Am J Clin Pathol;106(2):180-4; Gupta CK, Biologicals 1996;24(1):41-9; Gutierrez J, 1997;90, 151-4; Gratacap-Cavallier B; Eur J. Epidemiol 1998;14:147-52; Schmidt CA, Transplantation 1995;59(8):1133-8; Rosenthal SL, Clin Infect Dis 1997;24(2):135-9.

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.

Serum	Mean OD	Ab Index
Calibrator	1.84	
Positive Control	1.86	2.01
Negative Control	0.05	0.06
Reference Serum 1	1.94	2.09
Reference Serum 2	1.79	1.93
Reference Serum 3	2.01	2.17
Reference Serum 4	1.87	2.02
Negatives	0.17	0.18

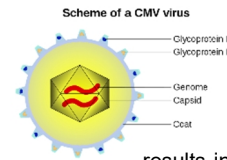
CALCULATION OF RESULTS

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the mean values of each sample by cut-off value.

Example of typical results:

Calibrator mean OD = 0.8
 Calibrator Factor (CF) = 0.5
 Cut-off Value = 0.8 x 0.5 = 0.400
 Positive control O.D. = 1.2
 Ab Index = 1.2 / 0.4 = 3
 Patient sample O.D. = 1.6
 Ab Index = 1.6 / 0.4 = 4.0



CMV is an enveloped dsDNA virus (~200 kb). CMV replicates in Epithelial cells, endothelial cells, fibroblasts and smooth muscle cells are predominant targets, but infects also parenchymal cells and connective tissue cells of virtually any organ and various hematopoietic cell types. During the lytic infection, viral immediate-early genes are expressed [8]. The expression of these genes results in the production of viral immediate-early proteins that modulate the host cell environment and stimulate the expression of viral early genes.

The enzyme-linked immunosorbent assay (or ELISA) is the most commonly available serologic test for measuring antibody to CMV. The result can be used to determine if acute infection, prior infection, or passively acquired maternal antibody in an infant is present. Other tests include various fluorescence assays, indirect hemagglutination, (PCR) and latex agglutination.

CMV Vaccines: As of 2015 no approved vaccine exists, although a number of vaccine candidates are under investigation. They include recombinant protein, live attenuated, DNA and other vaccines.

PRINCIPLE OF THE TEST

Alpha Diagnostic's Cytomegalovirus (CMV) IgM ELISA Kit is based on the principle of the enzyme immunoassay (EIA). Diluted patient serum is added to wells coated with purified CMV antigen. CMV IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRECAUTIONS

The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), Diluted H₂SO₄ (1N, stop solution), and Thimerosal (0.02% v/v in standards, conjugate diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:21 with ready-to-use sample diluent (e.g. 10 µL serum + 200 µL sample diluent).

REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:20 with water. (**Dilute 25 ml stock with 475 ml distilled water**) Store diluted buffer at 4°C for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute all samples 1:21 with the sample diluent. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. **DO NOT dilute calibrators or controls.**

1. Label or mark the microtiter well strips to be used on the plate. Prepare 1:21 dilution of unknowns, by adding 10 µl of the unknown to 200 µl of sample diluent. Mix Well.
2. Dispense 100 µl diluent in 1 well to be used as blank. Pipet **100 µl of , Prediluted controls, and samples** into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and **incubate at room temp for 20 min.**
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 µl of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 µl anti-IgM-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and **incubate for 20 minutes** at room temp (18-26°C).
5. **Wash the wells 3 times** as in step 3.
6. Add **100 µl TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and **incubate for 10 minutes** at room temp. Blue color develops in positive controls and samples.
7. Stop the reaction by adding **100 µl of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (**blue color turns yellow**).
8. **Measure the absorbance at 450 nm** using an ELISA reader within 60 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Do not touch the bottom of the wells.