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

Catalog#	Prod Description
1050	Monkey C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
2970	Monkey Circulating Immune complexes (CIC) ELISA Kit, 96 tests
3300-770-CMG	Monkey Anti-Cytomegalovirus (HCMV/CMV/Human Herpes Virus-5/HHV-5) IgG ELISA kit, 96 tests, Quantitative
3300-775-CMM	Monkey Anti-Cytomegalovirus (HCMV/CMV/Human Herpes Virus-5/HHV-5) IgM ELISA kit, 96 tests, Quantitative
3300-780-CMA	Monkey Anti-Cytomegalovirus (HCMV/CMV/Human Herpes Virus-5/HHV-5) IgA ELISA kit, 96 tests, Quantitative
3610-MKG	Monkey Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
4250	Monkey Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA kit, 96 tests, quantitative
4250-30-IGA	Monkey Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgA ELISA kit, 96 tests, quantitative
4350	Monkey Anti-Hepatitis B Surface Antigen Pres-S1 (HB-Pre-S1) IgG ELISA kit, 96 tests, quantitative
4355	Monkey Anti-Hepatitis B Surface Antigen Pres-S1 (HB-Pre-S1) IgM ELISA kit, 96 tests, quantitative
4450	Monkey Anti-Hepatitis B Surface Antigen Pres-S2 (HB-Pre-S2) IgG ELISA kit, 96 tests, quantitative
4455	Monkey Anti-Hepatitis B Surface Antigen Pres-S2 (HB-Pre-S2) IgM ELISA kit, 96 tests, quantitative
4550	Monkey Anti-Hepatitis B Surface Antigen Pres-S1+2 (HB-Pre-S1+2) IgG ELISA kit, 96 tests, quantitative
4555	Monkey Anti-Hepatitis B Surface Antigen Pres-S1+2 (HB-Pre-S1+2) IgM ELISA kit, 96 tests, quantitative
4580	Monkey Anti-Hepatitis B core (anti-HBcAg) IgG ELISA kit, 96 tests, Quantitative
4585	Monkey Anti-Hepatitis B core (anti-HBcAg) IgM ELISA kit, 96 tests, Quantitative
4620	Monkey anti-Hepatitis C virus (anti-HCV) IgG ELISA kit, 96 tests, Quantitative
4625	Monkey anti-Hepatitis C virus (anti-HCV) IgM ELISA kit, 96 tests, Quantitative
4670	Monkey Anti-Hepatitis A IgG (Anti-HAV) ELISA Kit, Quantitative, 96 tests
4675	Monkey Anti-Hepatitis A IgM (Anti-HAV) ELISA Kit, Quantitative, 96 tests
5030	Monkey Anti-Proliferating Cell Nuclear Antigen (PCNA) IgG ELISA kit, 96 tests, Quantitative
510-320-MRA	Monkey Anti-Respiratory syncytial virus F protein (RSV-F) IgA ELISA kit, 96 tests, quantitative
510-325-MRG	Monkey Anti-Respiratory syncytial virus F protein (RSV-F) IgG ELISA kit, 96 tests, quantitative
510-330-MRM	Monkey Anti-Respiratory syncytial virus F protein (RSV-F) IgM ELISA kit, 96 tests, quantitative
520-160-MMG	Monkey Anti-Mumps Virus (parotitis) IgG ELISA Kit, 96 tests, Quantitative
520-165-MMM	Monkey Anti-Mumps Virus (parotitis) IgM ELISA Kit, 96 tests, Quantitative
520-260-BVG	Monkey Anti-Varicella Zoster Virus (VZV/chickenpox) IgG ELISA kit, 96 tests, Quantitative
520-270-BVM	Monkey Anti-Varicella Zoster Virus (VZV/chickenpox) IgM ELISA kit, 96 tests, Quantitative
520-280-BVA	Monkey Anti-Varicella Zoster Virus (VZV/chickenpox) IgA ELISA kit, 96 tests, Quantitative
530-170-MMG	Monkey Anti-Measles IgG ELISA kit, 96 tests, Quantitative
530-180-MMM	Monkey Anti-Measles IgM ELISA kit, 96 tests, Quantiative
530-460-CEG	Monkey Anti-Chikungunya virus E1 (CHIKV-E1) IgG ELISA kit, 96 tests, Quantitative
530-470-CEM	Monkey Anti-Chikungunya virus E1 (CHIKV-E1) IgM ELISA kit, 96 tests, Quantitative
530-540-CEG	Monkey Anti-Chikungunya virus E2 (CHIKV-E2) IgG ELISA kit, 96 tests, Quantitative
530-550-CEM	Monkey Anti-Chikungunya virus E2 (CHIKV-E2) IgM ELISA kit, 96 tests, Quantitative
540-120-ENG	Monkey Anti-Dengue virus 1 Envelop protein IgG ELISA kit, 96 tests, quantitative
540-180-PRG	Monkey Anti-Dengue virus 1 prM protein IgG ELISA kit, 96 tests, quantitative
540-220-ENG	Monkey Anti-Dengue virus 2 Envelop protein IgG ELISA kit, 96 tests, quantitative
540-280-PRG	Monkey Anti-Dengue virus 2 prM protein IgG ELISA kit, 96 tests, quantitative
540-320-ENG	Monkey Anti-Dengue virus 3 Envelop protein IgG ELISA kit, 96 tests, quantitative

Instruction Manual No. M-3300-770-CMG

Monkey Cytomegalovirus (CMV) IgG ELISA KIT

Cat. # 3300-770-CMG, 96 Tests

For the detection of IgG antibody to Cytomegalovirus (CMV) in monkey serum or plasma

For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

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Web Site: www.4adi.com

Kit Components (96 tests)	
CMV antigen coated strip plate, (8x12 strip or 96 wells) # 3300-770-P	1 plate
CMV IgG **Calibrator,** 1 ml # 3300-770-1, yellow cap	1 vial
CMV IgG **Positive Control**, 1 ml # 3300-770-2, red cap	1 vial
CMV IgG **Negative Control**, 1 ml 3300-770-3, blue cap	1 vial
Anti-Monkey IgG-HRP Conjugate, (12 ml) # 3300-770-4	1 bottle
Sample Diluent, 22 ml # 3300-770-5	1 bottle
Wash buffer (20X) 25 ml # 3300-770-WB	1 bottle
TMB Substrate Solution, 12 ml # 3300-770-TMB	1 bottle
Stop Solution, 12 ml # 3300-770-ST	1 bottle
Complete Instruction Manual	M-3300-770-CMG

Intended Use

ADI Monkey Cytomegalovirus (CMV) IgG ELISA Kit is intended for the detection of IgG antibody to CMV virus in monkey serum or plasma. This kit is for in vitro research use only (RUO).

Introduction

Cytomegalovirus (CMV) (from the Greek cyto-, "cell," and megalo-, "large") is a genus of viruses in the order Herpesvirales, in the family Herpesviridae in the subfamily Betaherpesvirinae. Humans and monkeys serve as natural hosts. The eight species in this genus include the type species, Human betaherpesvirus 5 (HCMV, human cytomegalovirus, HHV-5), which is the species that infects humans. Diseases associated with HHV-5 include mononucleosis, and pneumonia. In the medical literature, most mentions of CMV without further specification refer implicitly to human CMV. Human CMV is the most studied of all cytomegaloviruses

Cytomegalovirus is a genus of enveloped DNA viruses that infect humans and monkeys. One species is clinically relevant, human cytomegalovirus HHV-5, that is simply referred to as cytomegalovirus. This pathogen is associated with mononucleosis and pneumonia, as well as infections in immunocompromised individuals and newborn.

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 IgG 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 IgG antibody to CMV.

0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1 Detectable IgG antibody to CMV.

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 diptheriae.

References: Mangano WE (1996) Am J Clin Pathol;106(2):180-4; Gupta CK, Biologicals 1996;24(1):41-9; Guti´errez 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.

WORKSHEET OF A TYPICAL ASSAY

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.

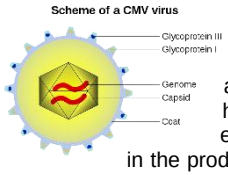
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.

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 . 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) IgG 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 IgG 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 IgG 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 IgG 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 4oC 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^{o}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^{o}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 ul of the unknown to 200 ul of sample diluent. Mix Well.

2. Dispense **100 ul** sample diluent in 1 well to be used as blank. Pipet **100 ul 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 ul 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 ul anti-IgG-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 (18-26oC).

5. Wash the wells 3 times as in step 3.

6. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **10 minutes** at room tempr. Blue color develops in positive controls and samples.

7. Stop the reaction by adding **100 ul 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^{o}C. Do not touch the bottom of the wells.