

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
970-100-PHG	Human Anti-Polio Virus IgG ELISA kits, 96 tests, Quantitative
540-110-DHM	Human Anti-Polio 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-Vacmune/Immucoteth (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-510-235-HEG

Human Anti-Epstein Barr Virus (EBV/HHV-4) virus capsid antigen (VCA) IgG ELISA Kit

Cat. # 510-235-HEG

**For the detection of IgG class antibodies against EBV
VCA in human serum or plasma.**

For In Vitro Research Use Only



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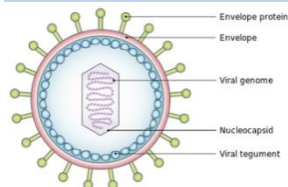
Web Site: www.4adi.com

Kit Components (96 tests)	
EBV VCA antigens coated strip plate, (8x12 strip or 96 wells) # 510-236P	1 plate
Calibrator A (2 mL; Negative control) #510237A	1 vial
Calibrator B (3 mL; Cut-off standards) #510237B	1 vial
Calibrator C (2 mL; Positive control) #510237C	1 vial
Anti-Human IgG-HRP Conjugate, (20 ml) #510238	1 bottle
Sample Diluent, 100 ml # 510239	1 bottle
Wash buffer (20X) 50 ml # 510235-WB	1 bottle
TMB Substrate Solution, 15 ml #510235-TMB	1 bottle
Stop Solution, 15 ml # 510235-ST	1 bottle
Complete Instruction Manual, M-510-235-HEG	1

Intended Use

ADI Human Anti-Epstein Barr virus EBV VCA IgG ELISA Kit is intended for the detection of IgG antibody against EBV VCA in human serum or plasma. Vaccine status of humans can also be assessed. **This kit is for in vitro research use only.**

General Information



The Epstein-Barr virus (EBV), also called human herpesvirus 4 (HHV-4), is a virus of the herpes family, and is one of the most common viruses in humans. It is the cause of infectious mononucleosis (glandular fever). It is also associated with particular forms of cancer, such as Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and conditions associated with human immunodeficiency virus (HIV) such as hairy leukoplakia

and central nervous system lymphomas. There is evidence that infection with the virus is associated with a higher risk of certain autoimmune diseases, especially dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and multiple sclerosis. Infection with EBV occurs by the oral transfer of saliva and genital secretions. Most people become infected with EBV and gain adaptive immunity. In the United States, about half of all five-year-old children and 90 to 95 percent of adults have evidence of previous infection. Infants become susceptible to EBV as soon as maternal antibody protection disappears. When infection with EBV occurs during adolescence, it causes infectious mononucleosis 35 to 50 percent of the time. The Epstein-Barr virus vaccine is not yet available. Gp350/220 and MVA-EL have been proposed as a target.

EBV can be divided into two major types, EBV type 1 and EBV type 2. These two subtypes have different EBNA-3 genes. As a result, the two subtypes differ in their transforming capabilities and reactivation ability. Type 1 is dominant throughout most of the world, but the two types are equally prevalent in Africa. EBV virus is approximately 120 nm to 180 nm in diameter and is composed of a double helix of DNA wrapped in a protein capsid. The capsid is surrounded by a tegument made of protein, which in turn is surrounded by a

SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

Precision :

Intraassay	n	Mean (E)	CV (%)
#1	24	0.485	5.90
#2	24	1.442	5.31
#3	24	1.558	1.97

Interassay	n	Mean (U)	CV (%)
#1	12	29.15	5.21
#2	12	31.99	7.22
#3	12	5.62	9.67

Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100% (95% confidence interval: 90.26% - 100%).

Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100% (95% confidence interval: 97.52% - 100%).

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

LIMITATIONS OF THE TEST

- 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. 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.
- 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 Good Laboratory Practices (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.

CALCULATION OF RESULTS

Run Validation Criteria:

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank:** Absorbance value < 0.100
- **Negative Control:** Absorbance value < 0.200 and < Cut-off
- **Cut-off Control:** Absorbance value 0.150 – 1.300
- **Positive Control:** Absorbance value > Cut-off

CALCULATION OF RESULTS

If these criteria are not met, the test is not valid and must be repeated.

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control

$$0.42 = 0.86 / 2 = 0.43$$
$$\text{Cut-off} = 0.43$$

Results in Units [U]

$\frac{\text{Sample (mean) absorbance value}}{\text{Cut-off}} \times 10 = [\text{Units} = \text{U}]$

Example: $\frac{1.591}{0.43} \times 10 = 37 \text{ U (Units)}$

Interpretation of Results

These values are derived from human samples to be used as general guidance as there is no monkey specific data available.

Cut-off	Negative	Equivocal	Positive
10 U	<9 U	9-11 U	>11 U

References: Maeda E (2009) Jpn. J. Radiol. 27, 4-19; Toussiot E (2008) Best Practice & Research. Clinical Rheumatology 22 (5): 883-96; Anthony EM (1964) Lancet 1, 702-703; Henle W (1980) Ann. NY Acad. Sci. 354, 326-331; Lerner AM (2004) In Vivo 18, 101-106; Fleisher G (1983) J. Inf. Dis. 147, 982-986;

envelope made from lipids. The viral envelope contains glycoproteins, which are essential to infection of the host cell. Epstein-Barr nuclear antigen 1 (EBNA1) is a multifunctional, dimeric viral protein associated with Epstein-Barr virus (EBV). It is the only EBV protein found in all EBV-related malignancies.

EBV infection may be identified by PCR and by the presence of antibodies (IgG, IgM and IgA) by ELISA. The optimal combination of serologic testing consists of the titration of four markers: IgM and IgG to the viral capsid antigen (VCA), IgM to the early antigen, and antibody to EBV nuclear antigen (EBNA). IgM to VCA appears early in infection and disappears within 4 to 12 weeks. IgG to VCA appears in the acute phase, peaks at 2 to 4 weeks after onset, declines slightly, and then persists for life. If antibodies to the viral capsid antigen are not detected, the patient is susceptible to EBV infection.

The optimal combination of serologic testing consists of the titration of four markers: IgM and IgG to the viral capsid antigen (VCA), IgM to the **early antigen (EA)**, and antibody to EBV nuclear antigen-1 (**EBNA-1**). IgM to VCA appears early in infection and disappears within 4 to 12 weeks. IgG to VCA appears in the acute phase, peaks at 2 to 4 weeks after onset, declines slightly, and then persists for life. Anti-EA IgG appears in the acute phase of illness and generally falls to undetectable levels after 3 to 6 months. In many people, detection of antibody to EA is a sign of active infection. If antibodies to the viral capsid antigen are not detected, the patient is susceptible to EBV infection.

No approved **EBV vaccine** currently available. Several vaccines using EBV Gp350/220 and MVA-EL (modified vaccine Ankara-expressing EBV antigens: 280-aa from the C-terminus of EBNA1 and the full 497-aa LMP2A fusion proteins) are in clinical trials.

PRINCIPLE OF THE TEST

ADI's EBV VCA IgG antibody ELISA Kit is based on the principle of the enzyme immunoassay (EIA). EBV VCA antigen is bound on the surface of the microtiter strips. Diluted unknowns are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized EBV VCA antibody takes place. Diluted patient serum is added to wells coated with purified. 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 that produced blue color. 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

Only for in-vitro use! 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. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. 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. 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.

MSDS

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site (www.4adi.com)

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample 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 1:100 with ready-to-use sample diluent (e.g. 5 µL serum + 495 µL sample diluent). Do not dilute the calibrators.

REAGENTS PREPARATION

1. **Dilute Wash buffer (20X)** 1:19 with distilled water. (e. g. **10 mL Washing Buffer + 190 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 1:100 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. **Dilute wash buffer stock (10X) 1:10 with distilled water.**

1. Label or mark the microtiter well strips to be used on the plate
2. Dispense **100 µl** diluent in 1 well to be used as blank. Pipet **100 µl of , calibrators, controls, and diluted samples (100:101)** 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 (37°C) for 60 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-human 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 30 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 20 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.