## 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 (chikenpox) IgG ELISA, 96 tests, Quantitative |
| 520-210-HVM | Human Anti-Varicella Zoster Virus (chikenpox) IgM ELISA, 96 tests, Quantitative |
| 520-220-HVG | Human Anti-Varicella Zoster Virus (chikenpox) 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/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 | Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgG ELISA Kit, 96 tests |
| AE-320430-1 | Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgM ELISA Kit, 96 tests |
| AE-320520-1 | Human Anti-Zaire-Ebola virus IgG ELISA Kit, 96 tests |



*Instruction Manual No. M*-510-245-EDG

# Human Anti-Epstein Barr Virus Early Antigen (EA) IgG ELISA Kit

# Cat. # 510-245-EDG

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

*For In Vitro Research Use Only*

4638 N Loop 1604 West • San Antonio• Texas 78249 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: Techsupport@4adi.com

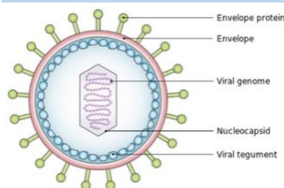
Web Site: www.4adi.com

| Kit Components (96 tests) | |
|---|---|
| EBV EA antigen coated strip plate, (8x12 strip or 96 wells) # 510-246P | 1 plate |
| **Standard serum** (2 mL) #510247A, ready-to-use | 2 vials |
| Negative **Standard serum** (2 mL) #510247B | 1 vial |
| Anti-Human **IgA, IgM or IgG-HRP Conjugate**, (13 ml) #510248 | 1 bottle |
| **Sample Dilution buffer**, 50 ml # 510249 | 2 bottles |
| **Wash buffer** (30X) 33 ml # 510245-WB | 1 bottle |
| **Substrate Solution**, 13 ml #510245-TM | 1 bottle |
| **Stop Solution**, 15 ml # 510245-ST | 1 bottle |
| Complete Instruction Manual, M-510-245-EDG | 1 |

## Intended Use

ADI Human Anti-Epstein Barr virus EBV EA IgG ELISA Kit is a qualitative immunoassay intended for the detection of IgG antibody against EBV EA 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.

### Sensitivity and Specificity

The evaluation of ELISA Epstein-Barr Virus EA IgG was verified in an internal study. The sensitivity was 96.9% and the specificity was 81.8%Borderline results were not included in the calculation of performance characteristics

### Interferences

To determine the influence of interfering substances, sera with different reactivities were analyzed with ELISA Epstein-Barr Virus EA IgG. No interferences have been detected for sera with concentrations up to 2.00 g/L hemoglobin, 11.50 g/L lipemia/triglyceride or 0.201 g/L bilirubin (conjugated and unconjugated).

### Cross Reactivity

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with ELISA Epstein-Barr Virus EA IgG and a commercially available anti-EBV EA IgG ELISA. Positive sera (10 sera each) for Herpes Simplex Virus IgG, Cytomegalovirus IgG, Rubella Virus IgG, Toxoplasma gondii IgG and Varicella Zoster Virus IgG have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation potential cross-reactivities with one Cytomegalovirus IgG, four RF and three ANA positive serum samples have been observed. Other cross-reactivities cannot be ruled out in general.

## 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 **< 0.25 OD**

The negative control must produce a negative test result. The mean OD-value (after subtraction of the substrate blank!) of the standard serum must be within the validity range, which is given on the lot specific quality control certificate. The variation of OD-values of the standard serum may not be higher than 20%.

**CALCULATION OF RESULTS**

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

**Cut-off Calculation:**

A lot specific quality control certificate is included in the test kit so that the obtained OD values can be interpreted qualitatively. The substrate blank must be subtracted from all OD values prior to evaluation. To fix the cut-off ranges multiply the mean value of the measured standard OD with the lot specific correction factor from the quality certificate. Then add and subtract the lot specific grey zone percentage mentioned on the quality certificate to obtain the upper and lower cut-off. The following numbers are an example only, Lot specific correction factor: 0.805Lot specific grey zone: 15%If the measured mean absorbance value of the standard serum is 0.84 OD, the range of the cut-off is: Lower cut-off: (0.84 * 0.805) -15% = OD 0.575Upper cut-off: (0.84 * 0.805) +15% = OD 0.778

**Borderline Ranges:**

The borderline range indicates the range for borderline test results. Values obtained, when testing a sample, which fall below this range indicate a negative test result; values above the borderline range are interpreted positive. In cases where the results are within the borderline range a definitive interpretation of the result is not possible. In such cases, the test should be repeated in parallel with a follow-up sample taken one to two weeks later (serum pair).

**REFERENCES**:


1. Aalto, S. M. (1998) Immunoreactivation of Epstein-Barr Virus Due to Cytomegalovirus Primary Infection. J. Med. Virol. *56*, 186 – 91.

2. Bauer, G. (2001) Simplicity Through Complexity: Immunoblot with Recombinant Antigens as the New Gold Standard in Epstein-Barr Virus Serology. Clin. Lab. *47*,223 – 30.

3. Dobson, R,, Topping, J., Giovannoni, G. (2013) Comparison of two commercial ELISA systems for evaluating anti-EBNA1 IgG titers. J. Med. Virol. *85*, 128 – 31.




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 EA IgG antibody ELISA Kit is based on the principle of the enzyme immunoassay (EIA). EBV EA 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 EA 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 Prolcin-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 (30X)** 1:30 with distilled water. (**Final vol of 1000mL 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.***

## Dilution of Samples

**Before running the test, samples (V1) must be diluted in dilution buffer (V2) as follows:**
**V1 + V2 = 1+ 100 add 10 µl sample dilution buffer each to 1000 µl**

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

Note: Dilution buffer for Epstein-Barr Virus EA IgG **is not exchangeable against the dilution buffer for other ELISA kits**

## 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 1:100 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 ul** diluent in 1 well to be used as blank. Pipet **100 ul of ,** negative control/standard serum, 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 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-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 (**20-25°C**) in the dark.
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 **30 minutes** at room temp. **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.