

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-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-510-205-HEG

Human Anti-Epstein Barr Virus (EBV/HHV-4) Nuclear Antigen 1 (EBNA-1) IgG ELISA Kit

Cat. # 510-205-HEG

For the detection of IgG class antibodies against EBV EBNA-1 in human serum or plasma.

For In Vitro Research Use Only



**ALPHA DIAGNOSTIC
INTERNATIONAL**

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400

Fax: +91-11-42208444

Email: customerservice@atzlabs.com

Web: www.atzlabs.com



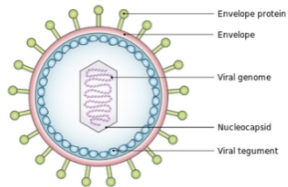
DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.

| Kit Components (96 tests) | |
|--|----------|
| EBV EBNA-1 antigens coated strip plate, (8x12 strip or 96 wells) # 510-206P | 1 plate |
| Calibrator A (2 mL; Negative control) #510207A | 1 vial |
| Calibrator B (2 mL; Cut-off standards) #510207B | 1 vial |
| Calibrator C (2 mL; Weak positive control) #510207C | 1 vial |
| Calibrator D (2 mL; Positive control) #510207D | 1 vial |
| Calibrator values are lot specific and specified on the vials. They are typically in the range of 0, 10, 50, and 150 U/ml for Calibrators A, B, C, and D respectively. | |
| Anti-Human IgG-HRP Conjugate , (15 ml) #510208 | 1 bottle |
| Sample Diluent , 60 ml # 510209 | 1 bottle |
| Wash buffer (10X) 60 ml # 510205-WB | 1 bottle |
| TMB Substrate Solution, 15 ml #510205-TMB | 1 bottle |
| Stop Solution , 15 ml # 510205-ST | 1 bottle |
| Complete Instruction Manual, M-510-205-HEG | 1 |

Intended Use

ADI Human Anti-Epstein Barr (EBNA-1) IgG ELISA Kit is intended for the detection of IgG antibody against EBV EBNA-1 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

LIMITATIONS OF THE TEST

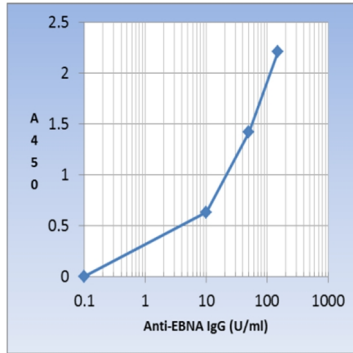
- The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized 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 and

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;

WORKSHEET OF A TYPICAL ASSAY

| Wells | Stds/samples | Mean A450 | Results |
|--------|--|-----------|----------|
| A1, A2 | Calibrator A Negative Control (0 U/ml) | 0.005 | |
| B1, B2 | Calibrator B Cut-off standard (10 U/ml) | 0.633 | |
| C1, C2 | Calibrator C Weak Positive (50 U/ml) | 1.426 | |
| D1, D2 | Calibrator D Positive Control (150 U/ml) | 2.214 | |
| E1, E2 | Sample 1 | 0.40 | Negative |

NOTE: These data are for **demonstration purpose only**. Use the values that are generated with each test.



Graph/510-205-HEG/6-ADI

CALCULATION OF RESULTS

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

The OD of the calibrators (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.

The initial dilution of unknowns has been taken into consideration when reading the results from the graph. Results of unknowns of higher predilution have to be adjusted for the dilution factor.

Unknowns showing concentrations above the highest calibrator have to be diluted as described in "Assay Procedure" and reassayed.

Values suggested by manual procedure:

| EBNA IgG (U/mL) | Results |
|-----------------|-----------|
| <8 | negative |
| 8-12 | equivocal |
| >12 | positive |

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 EBNA-1 antibody ELISA Kit is based on the principle of the enzyme immunoassay (EIA). EBV EBNA-1 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 EBNA-1 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.

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

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** 1:10 with water. (**Dilute 60 ml stock with 940 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 ul** diluent in 1 well to be used as blank. Pipet **100 ul 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 (25-28°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 (18-26°C).
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 20 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°C. Do not touch the bottom of the wells.