

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

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

## Human Anti-Herpes Simplex Virus 2 IgG (HSV-2 IgG) ELISA KIT

**Cat. # 3300-390-H2G**

**For the detection of IgG antibody to HSV-2 in Human serum or plasma**

*For In Vitro Research Use Only (RUO)*



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Kit Components (96 tests)	
HSV-2 antigen coated strip plate, (8x12 strip or 96 wells) # 3300-390-P	1 plate
Calibrator A (Negative Control), 2 ml # 3300-390-1	1 vial (Blue cap)
Calibrator B (Cut-Off Standard), 3 ml # 3300-390-2	1 vial (Green cap)
Calibrator C (Positive Control), 2 ml # 3300-390-3	1 vial (Red cap)
Anti-Human IgG-HRP Conjugate, (20 ml) # 3300-390-5	1 bottle
Sample Diluent, 100 ml # 3300-390-5	1 bottle
Wash buffer (20X), 50 ml # 3300-390-WB	1 bottle
TMB Substrate Solution, 15 ml # 3300-390-TMB	1 bottle
Stop Solution, 15 ml # 3300-390-ST	1 bottle
Complete Instruction Manual	M-3300-390-H2G

### Intended Use

ADI Human Herpes Simplex Virus 2 IgG (HSV-2) IgG ELISA Kit is intended for the detection of IgG antibody to HSV-2 virus in Human serum or plasma. This kit is **for in vitro research use only (RUO)**.

### Introduction

HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they are teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation, and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure A significant increase in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.

### Evaluation (U/mL)

The ready-to-use standards and controls of the HSV-2 IgG antibody kit are defined and expressed in arbitrary units (U/mL). Consequently, for a given subject follow-up controls become possible. For this evaluation, the absorptions of the standards and controls are graphically drawn point-to-point against their concentrations. From the resulting reference curve, the concentration values for each sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit point-to-point must be chosen. Calibrator B with its concentration of 10 U/mL serves as cut-off standard. Analogous to the cut-off evaluation a range of +/-20% around the cut-off is defined as a grey zone. Thus, results between 8 and 12 U/mL are reported as borderline.

### ASSAY CHARACTERISTICS

HSV-2 ELISA	IgG
Intra-Assay-Precision	9.3 %
Inter-Assay-Precision	7.6 %
Inter-Lot-Precision	3.3 – 8.8 %
Analytical Sensitivity	0.98 U/mL
Recovery	97 – 114 %
Linearity	81 – 107 %
Cross-Reactivity	No cross-reactivity to Measles, Mumps and Varicella.
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
<b>Specificity</b>	<b>97.76 %</b>
<b>Sensitivity</b>	<b>99.31 %</b>

## EVALUATION (Cut-Off)

The calculated absorptions for the sera, are compared with the value for the cutoff standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same subject, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

### 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

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]

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

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

### Evaluation of Results

10 U	Cut-off-
> 11 U	Antibodies against the antigen are present.
9 – 11 U	Antibodies against the antigen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
< 9 U	The sample contains no antibodies against the antigen.

## PRINCIPLE OF THE TEST

Alpha Diagnostic hsv-2 IgG ELISA Kit is based on the principle of the enzyme immunoassay (EIA). Diluted serum or ready-to-use standards are added to wells coated with HSV-2 antigen. HSV-2 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 H<sub>2</sub>SO<sub>4</sub> (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 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

### 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 1:101 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 1:101 dilution of unknowns, by adding 5 ul of the unknown to 500 ul of sample diluent. Mix Well.
2. Dispense **100 ul** sample diluent in 1 well to be used as blank. Pipet **100 ul of ready-to-use standards and controls, diluted samples** into appropriate wells in *duplicate*. Cover the plate, mix gently for 5-seconds and **incubate at 37 °C temp 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-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°C to 25°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 in the dark. 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.