

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

Catalog#	ProdDescription
510-100-HRG	Human Anti-Rubella Virus IgG ELISA kit, 96 tests, quantitative
510-110-HRM	Human Anti-Rubella Virus IgM ELISA kit, 96 tests, quantitative
510-120-MRG	Mouse Anti-Rubella Virus IgG ELISA kit, 96 tests, quantitative
510-130-MRM	Mouse Anti-Rubella Virus IgM ELISA kit, 96 tests, quantitative
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
520-130-MMG	Mouse Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
520-140-MMM	Mouse Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative
520-150-MMA	Mouse Anti-Mumps Virus (parotitis) IgA ELISA, 96 tests, Quantitative
530-100-HMG	Human Anti-Measles IgG ELISA kit, 96 tests, Quantitative
530-110-HMM	Human Anti-Measles IgM ELISA kit, 96 tests, Quantitative
530-120-HMA	Human Anti-Measles IgA ELISA kit, 96 tests, Quantitative
530-130-MMG	Mouse Anti-Measles IgG ELISA kit, 96 tests, Quantitative
530-140-MMM	Mouse Anti-Measles IgM ELISA kit, 96 tests, Quantitative
530-150-MMA	Mouse Anti-Measles IgA ELISA kit, 96 tests, Quantitative
960-110-PHG	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG, 96
960-120-PHG	Mouse Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA
960-130-PMG	Mouse Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-140-PHM	cat# changed to #960-140-PMM; Mouse Anti-B. pertussis IgM ELISA kit
960-140-PMM	Mouse Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-150-PRG	Rabbit Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-160-PRM	Rabbit Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-170-PMG	G. pig Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-180-PMM	G. pig Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-190-PHG	Human Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-195-PHM	Human Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-200-PHA	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA
960-205-PHA	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA
960-210-PHG	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA
960-220-PHM	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA
960-225-PHM	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA
960-230-PGG	Mouse Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-240-PRG	Rabbit Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-250-PHG	Human Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-260-PMG	Monkey Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-300-FMG	Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-310-FMM	Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96
960-320-FRG	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-330-FRM	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96
960-340-FHG	Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit,
960-350-FHM	Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit,
FHA11-S	Anti-B. pertussis Filamentous hemeagglutinin (FHA) protein antiserum
FHA15-N-10	Filamentous Hemeagglutinin (FHA) (B. pertussis), purified
FHA21-S	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM negative
FHA22-S	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM positive
FHA31-S	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG negative
FHA32-S	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG positive
FIM235-N-10	Purified FIMBRIAE 2/3 (Fim2/3) (B. pertussis), antigen grade
MESL11-A	Anti-Measles (Rubeola/Edmonston strain) Virus IgG
MESL12-M	Monoclonal Anti-Measles (Rubeola/Edmonston strain) Virus IgG
MESL15-N-500	Measles (Rubeola) Virus (Edmonston) proteins/antigen extract
MUMS11-S	Anti-Mumps virus (Enders) Virus antiserum
MUMS11-SB	Anti-Mumps virus (Enders) Virus antiserum
MUMS12-M	Monoclonal Anti-Mumps virus (Enders) Virus IgG

*Instruction Manual No. M-3300-380-H1G*

## **Human Anti-Herpes Simplex Virus 1 IgG (HSV-1 IgG)**

### **ELISA KIT Cat. # 3300-380-H1G**

For detecting human IgG antibodies against Herpes simplex  
1 (HSV-1 IgG) in serum and plasma

*For In Vitro Research Use Only (RUO)*



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

Human Anti-Herpes Simplex Virus 1 IgG ELISA KIT Cat. # 3300-380-H1G (96 tests)

Kit Components (96 tests)	
Herpes 1 IgG antigen coated strip plate, (8x12 strip or 96 wells) # 3300-381	1 plate
Herpes IgG calibrator A (Negative Control) , 1 mL #3300-382A	1 vial
Herpes IgG calibrator B (Cut-Off std) , 1 mL #3300-382B	1 vial
Herpes IgG calibrator C (Weak Positive control) , 1 mL #3300-382C	1 vial
Herpes IgG calibrator D (Positive control) , 1 mL #3300-382D	1 vial
All controls contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative	
Anti-Human IgG-HRP Conjugate, (15 ml) #3300-383	1 bottle
Sample Diluent, 60 ml #3300-380SD	1 bottle
Wash buffer (10X) 60 ml #3300-380WB	1 bottle
TMB Substrate Solution, 15 ml #3300-380-TM	1 bottle
Stop Solution, 15 ml # 3300-380ST	1 bottle
Re-sealable bag for the un-used antigen strips	1
Complete Instruction Manual, M-3300-380-H1G	1

**Intended Use:**

ADI Herpes simplex 1 IgG Antibody ELISA Test Kit has been designed for the detection of specific IgG antibodies against Herpes 1 in serum and plasma. For in vitro research use only (RUO).

**Introduction:**

The Herpes simplex virus type 1 is an ubiquitous pathogen of humans that usually causes either asymptomatic infection or mild skin and mucosal diseases. Antibodies to HSV 1 occur in about 90% of adults. Normally HSV 1 is transmitted by oral secretions or open wounds prior to the age of five. Recently in adults primary infections were observed, too. After the primary infection some viruses establish a latent state in their host cells (mostly ganglionic cells). The virus DNA is integrated into the genome of the host cell, where it remains until the infected person dies. After stimulation of the host cell, recurrent infection occurs, which is called an exacerbation, when clinical symptoms appear. The recurrence may be caused by different kinds of traumas, as fever or physiological changes and diseases. Immunosuppressed persons may show a severe clinical course. HSV 1 causes different symptoms in about 10% of the primary infections. HSV 1 causes 85% and HSV 2 15% of oral primary infections. The major manifestations associated with HSV 1 infections are gingivostomatitis, keratitis, conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and some lethal infections of newborns. In some cases HSV 1 infection leads to a meningitis with different neurological symptoms. Persons at an increased risk for serious or prolonged HSV infections are those with eczema, severe burns or a defect in their cell-mediated immunity. The drug Acyclovir is the treatment of choice for most serious HSV infections.

**Interpretation of Results**

U/mL	Interpretation
< 8	negative
8 – 12	equivocal
> 12	positive

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

**Expected Values**

In an in-house study apparently healthy subjects showed the following results:

Ig Isotype	n	Interpretation		
		Positive	Equivocal	Negative
IgG	88	69.3 %	3.4 %	27.3 %

**Quality Control**

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. The positive control must show at least double the OD of the cut-off standard. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

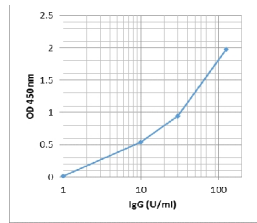
**PERFORMANCE CHARACTERISTICS:**

<b>Intra-Assay-Precision</b>	8.3 %
<b>Inter-Assay-Precision</b>	9.4 %
<b>Inter-Lot-Precision</b>	5.2 – 11.8 %
<b>Analytical Sensitivity</b>	1.10 U/mL
<b>Recovery</b>	90- 95 %
<b>Linearity</b>	73 – 117 %
<b>Cross-Reactivity</b>	No cross-reactivity to Measles, Mumps and Varicella
<b>Interferences</b>	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>Clinical Specificity</b>	96 %
<b>Clinical Sensitivity</b>	100 %

## WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Results
A1, A2	Calibrator A Negative Control (1 U/ml)	0.012	
B1, B2	Calibrator B Cut-off standard (10 U/ml)	0.539	
C1, C2	Calibrator C Weak Positive (40 U/ml)	0.942	
D1, D2	Calibrator D Positive Control (150 U/ml)	1.974	
E1, E2	Sample 1	0.415	Negative
F1, F2	Sample 1	1.62	Positive

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results. Lot specific values are provided on the vials and must be used for standard curve.



4-Arif-ELISA-Graphs

### CALCULATION OF RESULTS:

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

Examples: Blank 0.022

The obtained OD of the standards (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. For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read from the standards curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in "Test Procedure" and reassayed.

## PRINCIPLE OF THE TEST

Alpha Diagnostic's Herpes 1 IgG antibody test kit is based on the principle of the indirect ELISA. Herpes 1 virus antigens are bound on the surface of the microtiter strips. Diluted serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Herpes 1 antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. 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! Do not ingest or swallow! 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 \(substrate\), H2SO4 \(stop solution\), and Prolcin-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](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 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

#### REAGENTS PREPARATION:

1. **Dilute Wash buffer** 1:10 with 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). Dilute the wash buffer with water (1:10).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:101 (5 ul samples in 500 ul sample diluent)**. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **100 ul of Prediluted controls, and samples** (diluted 1:101) 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 for 60 min**.
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 250-300 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 (25-28°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.