

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

Instruction Manual No. M-3300-405-12M

Catalog#	Description
3300-370-CMG	Human Anti-Cytomegalovirus (HCMV/CMV) IgG ELISA kit, 96 tests, Quantitative
3300-375-CMM	Human Anti-Cytomegalovirus (HCMV/CMV) IgM ELISA kit, 96 tests, Quantitative
3300-380-CMA	Human Anti-Cytomegalovirus (HCMV/CMV) IgA ELISA kit, 96 tests, Quantitative
3300-380-H1G	Human Anti-Herpes Simplex Virus 1 IgG (HSV-1 IgG) ELISA kit, 96 tests,
3300-385-H1M	Human Anti-Herpes Simplex Virus 1 IgM (HSV-1 IgM) ELISA kit, 96 tests,
3300-390-H2G	Human Anti-Herpes Simplex Virus 2 IgG (HSV-2 IgG) ELISA kit, 96 tests,
3300-395-H2M	Human Anti-Herpes Simplex Virus 2 IgM (HSV-2 IgM) ELISA kit, 96 tests,
3300-400-12G	Human Anti-Herpes Simplex Virus 1+2 IgG (HSV-1/2 IgG) ELISA kit, 96 tests,
3300-405-12M	Human Anti-Herpes Simplex Virus 1+2 IgM (HSV-1/2 IgM) ELISA kit, 96 tests,
3300-410-HTA	Human Anti-Toxoplasma IgA (Toxo-IgA) ELISA kit, 96 tests, Quantitative
3300-415-HTG	Human Anti-Toxoplasma IgG (Toxo-IgG) ELISA kit, 96 tests, Quantitative
3300-420-HTM	Human Anti-Toxoplasma IgM (Toxo-IgM) ELISA kit, 96 tests, Quantitative
3300-500-HCA	Human Anti-Chlamydia Trachomatis IgA ELISA kit, 96 tests, semi-Quantitative
3300-510-HCG	Human Anti-Chlamydia Trachomatis IgG ELISA kit, 96 tests, semi-Quantitative
3300-520-HCM	Human Anti-Chlamydia Trachomatis IgM ELISA kit, 96 tests, semi-Quantitative
3300-530-HCA	Human Anti-Chlamydia Pneumonia IgA ELISA kit, 96 tests, semi-Quantitative
3300-540-HCG	Human Anti-Chlamydia Pneumonia IgG ELISA kit, 96 tests, semi-Quantitative
3300-550-HCM	Human Anti-Chlamydia Pneumonia IgM ELISA kit, 96 tests, semi-Quantitative
3300-600-HAA	Human Anti-Candida albicans IgA ELISA kit, 96 tests, semi-Quantitative
3300-605-HAG	Human Anti-Candida albicans IgG ELISA kit, 96 tests, semi-Quantitative
3300-610-HAM	Human Anti-Candida albicans IgM ELISA kit, 96 tests, semi-Quantitative
3300-770-CMG	Monkey Anti-Cytomegalovirus (HCMV/CMV) IgG ELISA kit, 96 tests, Quantitative
3300-775-CMM	Monkey Anti-Cytomegalovirus (HCMV/CMV) IgM ELISA kit, 96 tests, Quantitative
3300-780-CMA	Monkey Anti-Cytomegalovirus (HCMV/CMV) IgA ELISA kit, 96 tests, Quantitative

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
960-150-PRG	Rabbit Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-170-PMG	G. pig Anti-B. pertussis toxin/toxoid IgG 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
960-320-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,

Human Anti-Herpes Simplex Virus 1 +2 IgM (HSV-1/2 IgM) ELISA KIT

Cat. # 3300-405-12M, 96 Tests

For detecting human IgM antibodies against Herpes simplex
1+2 IgM (HSV-1/2 IgM) in serum and plasma

For In Vitro Research Use Only (RUO)



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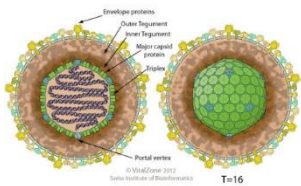
Web Site: www.4adi.com

Kit Components (96 tests)	
Herpes 1/2 IgM antigen coated strip plate, (8x12 strip or 96 wells) # 3300-406P	1 plate
Herpes 1/2 IgM calibrator A (Negative Control), 2 mL #3300-407A	1 vial (blue cap)
Herpes 1/2 IgM calibrator B (Cut-Off std), 3 mL #3300-407B	1 vial (green cap)
Herpes 1/2 IgM calibrator C (Positive control), 2 mL #3300-407C	1 vial (red cap)
All controls contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative	
Anti-Human IgM-HRP Conjugate, (20 ml) #3300-408	1 bottle
Sample Diluent, 100 ml #3300-405-SD	1 bottle
Wash buffer (20X) 50 ml #3300-405-WB	1 bottle
TMB Substrate Solution, 15 ml #3300-405-TM	1 bottle
Stop Solution, 15 ml # 3300-405-ST	1 bottle
Complete Instruction Manual, M-3300-405-12M	1

Intended Use:

Herpes simplex virus 1/2 IgM (HSV-1/2 IgM) Antibody ELISA Test Kit has been designed for the detection of specific IgM antibodies against Herpes 1/2 in serum or plasma or other biological fluids. **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 ganglial 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.

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:

Intra-Assay-Precision	4.2 %
Inter-Assay-Precision	7.9 %
Inter-Lot-Precision	5.2 – 11.1 %
Analytical Sensitivity	1.12 U/mL
Recovery	85- 93 %
Linearity	64 – 121 %
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
Clinical Specificity	100 %
Clinical Sensitivity	96.46 %

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

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]

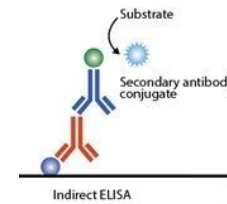
$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Units} = \text{U}]$

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

Evaluation of Results

Cut-Off	10 U	
Positive	> 11 U	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 U	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
Negative	< 9 U	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

PRINCIPLE OF THE TEST



Alpha Diagnostic's Herpes 1/2 IgM (HSV-1/2 IgM) antibody test kit is based on the principle of the enzyme immunoassay (EIA). Herpes 1/2 antigen is 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 IgM antibodies of the serum and the immobilized Herpes 1/2 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-IgM 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 IgM 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.

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). <http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf>

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). Dilute the wash buffer with water (1:20).

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 µl of Prediluted samples** (diluted 1:101) **ready to use standards & controls** 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 37°C 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-IgM-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 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 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.