

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
530-100-HMG	Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
530-110-HMM	Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative
530-120-HMA	Human Anti-Mumps Virus (parotitis) IgA ELISA, 96 tests, Quantitative
530-200-HVG	Human Anti-Varicella Zoster Virus (chickenpox) IgG ELISA, 96 tests, Quantitative
530-210-HVM	Human Anti-Varicella Zoster Virus (chickenpox) IgM ELISA, 96 tests, Quantitative
530-220-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-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 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-320530-1	Human Anti-Zaire-Ebola virus IgG ELISA Kit, 96 tests

Instruction Manual No. M-530-130-MMG

Mouse Anti-Measles IgG ELISA KIT

Cat. # 530-130-MMG, 96 Tests

For Detecting IgG antibodies against Measles Virus
In Mouse Serum or Plasma

For Research use only (RUO)



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)	qty
Measles antigen coated strip plate, (8x12 strip or 96 wells) # 530-131	1 plate
Measles IgG Calibrator A, (Negative Control), 2 mL # 530-132A	1 vial
Anti-Measles IgG Calibrator B, (Cut-Off) ,3 mL # 530-132B	1 vial
Anti-Measles IgG Calibrator C (Positive control), 2 mL #530-132C	1 vial
All calibrators contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative	
Anti-Mouse IgG-HRP Conjugate, 20 ml, H-MsG.6BL	1 bottle
Sample Diluent, 100 ml, #530-130-SD	1 bottle
Wash buffer (20X), 50 ml, #530-130-WB	1 bottle
TMB Substrate Solution, 15 ml, #530-130-TMB	1 bottle
Stop Solution, 15 ml, #530-130-ST	1 bottle
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Intended Use

Mouse Anti-Measles IgG ELISA Kit has been designed for the detection of IgG class antibodies against Measles in Mouse serum and plasma or other qualified biological samples from vaccinated, immunized and/or infected mice.

This immunoassay is suitable for:

- Determining **immune status** relative to non-immune controls.
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols.

The assay is for research use only (RUO) and is not intended for therapeutic uses.

Introduction

Measles is a highly contagious viral disease characterized by a clinically distinct prodrome of fever, coryza, conjunctivitis, cough and a pathognomic exanthem (Koplik's spots). The disease is the result of infection with the Measles Virus, genus Morbillivirus of the family Paramyxoviridae. Ten to twelve days after infection, the most prominent and characteristic prodromal symptoms appear: coryza; a persistent barking cough; keratoconjunctivitis, often with photophobia; and fever. Generally lymphadenopathy and splenomegaly are also frequent. During this period, Koplik's spots appear on the bucal mucosa that rapidly spread involving the entire mucous membrane. These spots are usually gone by the time the skin rash reaches its peak. The rash of Measles appears after a 3-to 5-days prodrome, some 14 days after exposure. The rash quickly becomes maculopapular and spreads rapidly over the face, neck, trunk and extremities during the next three days. At its height, the eruption has generally deepened to a reddish purple and may be associated with edema of the skin. Complications are: otitis media, pneumonia and encephalitis. Measles have a more severe expression in younger or undernourished children with a higher incidence of hemorrhage Measles, with 5% to 10% of lethal cases.

Interpretation of Results

The following data is derived from human samples.

U/mL	Interpretation
< 9	negative
9 – 11	equivocal
> 11	positive

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

Mouse Sample Testing

A panel of sera from non-vaccinated, adult mice (Balb/c, mixed sex) were tested in the assay at 1:100 dilution. A450 values ranged from 0.048 – 0.161. We recommend that users establish sample dilutions that will give acceptable basal values for their animals. The actual test dilution can be varied from 1:50-1:500. Mice with no exposure to measles should produce A450 values of <0.500 at a given dilution.

Expected Values

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

Ig Isotype	n	Interpretation		
		positive	equivocal	negative
IgG	48	93.8 %	0.0 %	6.2 %

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).

Run Validation Criteria

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.

WORKSHEET OF A TYPICAL ASSAY

Stds/samples	Mean A450
Blank (Standard A)	0.032
Standard B	0.499
Standard C	1.091

NOTE: **These data are for demonstration purpose only.** It must not be used to determine the sample results.

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 must be multiplied with the dilution factor. Samples showing concentrations above the highest standard must be diluted as described in "Test Procedure" and re-assayed.

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

$$0.43$$

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision 12.5 %
Inter-Assay-Precision 6.9 – 15.8 %
Sensitivity 75 – 129 %
Cross-Reactivity No cross-reactivity to Mumps and Varicella

Interferences Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin

Clinical Specificity 100 %, **Clinical Sensitivity** 97.01 %

Introduction (continued)

In people that have been vaccinated with inactive virus (before 1968), the infection can have severe manifestations as: pneumonia, peripheral edema, pleural effusion and atypical rash. Measles are one of the most contagious infectious diseases. The virus spreads through droplets emanating from the respiratory tract of infected persons or by direct contact. The incidence of Measles has declined since the introduction of vaccination programs.

In developed countries, most children are immunized against measles by the age of 18 months, generally as part of a three-part **MMR vaccine (measles, mumps, and rubella)**. In endemic countries, WHO recommend that two doses of vaccine be given at six months and at nine months of age. **MMR II** vaccine (Merck) is a live virus vaccine for vaccination against measles (rubeola), mumps, and rubella (German measles). Attenuated Measles virus, derived from Enders' attenuated Edmonston strain and propagated in chick embryo cell culture, is used in MMR II vaccine. This strain of virus was adapted for the hens egg and less well-suited for human cells. These strains are therefore called **attenuated** strains. The **MMRV vaccine**, a combined measles, mumps, rubella and varicella vaccine, has been proposed as a replacement for the MMR vaccine to simplify administration of the vaccines.

PRINCIPLE OF THE TEST

Alpha Diagnostic's Measles IgG antibody test kit is based on the principle of the indirect ELISA. Measles antigen is bound on the surface of the microtiter strips. Diluted patient 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 Measles antigen takes place. After a 60 minute incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Anti-IgG peroxidase conjugate is added and incubated. After a further washing step, the substrate (TMB) solution is pipetted and incubated, 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 at 450 nm in an ELISA reader. 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. Distilled water, reagent troughs, plate washer (recommended) and ELISA plate reader (450nm).

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 bromonitrodioxane (0.05% w/v in standards, sample diluent and HRP-conjugate).

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) will require dilution with sample diluent.

Mouse Sample Dilutions: An initial test dilution of 1:100 is recommended for most mice. Basal measles antibody values may differ due to strains, age, sex, and potential or intentional exposure to measles antigen. Therefore, final test dilution can be varied from 1:20-1:500. Vaccinated samples should be tested at higher dilutions or until sample values are within the range of the standards.

Sample Stability

Initial dilution of serum (1:10; or 5 ul serum into 45 ul diluent) into **Sample Diluent** is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for months stored frozen. Further test dilution (1:500 or more) should be performed within a week of the assay.

Example:

Initial (1:10): 5 ul serum + 45ul sample diluent (store frozen)

Further (1:100): 25 ul initial (1:10) + 225 ul sample diluent (use 100 ul x 2 for testing)

REAGENTS PREPARATION

Dilute Wash buffer 1:20 with water. (eg. 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.

Bring all reagents to room temperature prior to 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 12 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). Prepare Wash Buffer prior to start of assay.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag, sealed with desiccant. **All samples should be diluted (see page 3). Calibrator and control are provided ready to use.**

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **100 ul of sample diluent (buffer blank), Calibrators, controls, and samples** (diluted) into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Mix gently by tapping and **incubate at room temperature for 60 minutes**.
3. Aspirate (or dump) the well contents; immediately, **wash the wells 3 times** with 250-300 ul of 1X wash buffer, then blot the plate on absorbent paper. An automated ELISA plate Washer may be used for best consistency; manual washing may be most convenient for fewer strips. Failure to wash the wells properly may lead to high blank and sample values.
4. Add **100 ul anti-Mouse IgG-HRP conjugate** to all wells. Mix gently by tapping and **incubate for 30 minutes** at room temperature (18 - 28° C).
5. **Wash the wells 3 times** as in step 3.
6. Add **100 ul TMB substrate solution**. Mix gently as above and **incubate for 15 minutes** at room temperature. Blue color develops in positive controls and samples.
7. Stop the reaction by adding **100 ul of stop solution** to all wells. Tap gently for 5-10 seconds to have uniform color distribution (**blue color turns yellow**).
8. **Measure the absorbance at 450 nm** (using 620nm as reference is optional) using an ELISA reader within 60 minutes.

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 refrigerated in a sealed bag with desiccant. Do not touch the bottom of the wells.