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

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

Instruction Manual No. M-970-700-SMG

Human Anti-Schistosoma mansoni IgG ELISA Kit, Quantitative

Cat. # 970-700-SMG, 96 tests

For the detection of IgG antibody to Schistosoma mansoni in human serum or plasma

For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

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Kit Components (96 tests)	
Schistosoma mansoni antigen coated strip plate, (8x12 strip or 96 wells) # 970-701-P	1 plate
Negative Control, 2 ml 970-702A, blue cap	1 vial
Cut-off Control, 3 ml # 970-702B, green cap	1 vial
Positive Control, 2 ml # 970-702C, red cap	1 vial
HRP Conjugate, (20 ml) # 970-703	1 bottle
Sample Diluent, 100 ml # 970-700-SD	1 bottle
Wash buffer (20X) 50 ml # 970-700-WB	1 bottle
TMB Substrate Solution, 15 ml # 970-700-TMB	1 bottle
Stop Solution, 15 ml # 970-700-ST	1 bottle
Complete Instruction Manual, M- 970-700-SMG	1

Intended Use

ADI Human Anti-Schistosoma mansoni IgG ELISA Kit is intended for the detection of IgG antibody to Schistosoma mansoni in human serum or plasma (citrate, heparin). This kit is for **in vitro research use only (RUO).**

Introduction

Schistosoma mansoni is a water-borne parasite of humans and belongs to the group of blood flukes (Schistosoma). The adult lives in the blood vessels (mesenteric veins) near the human intestine. It causes intestinal schistosomiasis (similar to S. japonicum, S. mekongi, S. guineensis, and S. intercalatum). Clinical symptoms are caused by the eggs. As the leading cause of schistosomiasis in the world, it is the most prevalent parasite in humans. It is classified as a neglected tropical disease.

Schistosoma mansoni is common in Africa, South America and Middle East. Schistosomiasis (bilharziosis) is – depending on species and location of the parasites – a disease of the intestine, liver and spleen resp. urinary passages. Humans are (re)infected by contact with fresh water which is contaminated by ova containing urine or faeces. If larvae bore into human skin, first a transient skin reaction appears (itch with exanthema or erythema, by repeatedly infection cercarial dermatitis is possible). After 3 - 10 weeks the meanwhile sexually mature worms synthesize cytotoxic and allergic substances which course feverish reaction in humans (Katayama fever). The infected person is mostly harmed by the eggs, which get into organs via blood excreting proteins and glycoproteins. The person reacts under participation of own antibodies and immune complexes with formation of granuloma and granulomatous proliferation in intestine and urinary bladder mucosa. Not excreted eggs die after 3 weeks and will be dissolved or calcified. The affected tissue gets fibrous. In final stage bilharziosis will cause dead.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

- Substrate blank in A1: Absorbance value < 0.100.
- Negative control in B1: Absorbance value < 0.200 and < cut-off
- Cut-off control in C1 and D1: Absorbance value 0.150 – 1.300.
- Positive control in E1: Absorbance value > cut-off.

If these criteria are not met, the test is not valid and must be repeated.

INTERPRETATION

The following is intended as a guide to interpretation of Schistosoma mansoni IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

Samples are considered POSITIVE if the absorbance value is higher than 10% over the cut-off.
Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered NEGATIVE.
Samples are considered NEGATIVE if the absorbance value is lower than 10% below the cut-off.

LIMITATIONS OF THE TEST

1. 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.

2. Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values. In immune-compromised patients and newborns serological data only have restricted value.

Interferences

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

Performance Characteristics

Interassay: <10.71% Intraassay: <3.88%

Specificity: 98.55%

Sensitivity: 93.75%

WORKSHEET OF A TYPICAL ASSAY

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

Serum	Mean OD
Cut-off Control	0.488
Positive Control	0.967
Negative Control	0.003

CALCULATION OF RESULTS

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

Cut-off = 0.43

Results in Units:

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

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

Cut-off:	10 U
Grey zone:	9-11 U
Negative:	<9 U
Positive:	>11 U

PRINCIPLE OF THE TEST

Alpha Diagnostic's Human Anti-Schistosoma mansoni IgG ELISA Kit is based on the principle of the enzyme immunoassay (EIA). Diluted sample serum is added to wells coated with Schistosoma mansoni antigen. Schistosoma mansoni IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the HRP labelled conjugate is added to bind to the antibody-antigen complex, if present. Excess HRP labelled 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 using an ELISA microwell plate reader 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 micropipette (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. distilled 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 must 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 must 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 must be taken that the stoppers are not contaminated. Further a possible mix-up must 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 H2SO4 (1N, stop solution), and MIT (0.02% v/v in standards, conjugate diluent).

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (citrate, 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. Heat inactivation of samples is not recommended.

For the performance of the test the samples (not the controls) have to be diluted 1:100 with ready-to-use sample diluent (e.g. 10 µL serum + 1000 µL sample diluent).

REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:20 with water. (**Dilute Washing Solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh distilled water.**) Store diluted buffer at 4^{O}C for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°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^{O}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^{O}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 controls.

1. Label or mark the microtiter well strips to be used on the plate. Prepare 1:100 dilution of unknowns, by adding 10 ul of the unknown to 1000 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 controls, and diluted samples into appropriate wells in *duplicate*. Cover the plate, mix gently for 5-seconds, and incubate at 37±1°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 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. (20-25°C). Do not expose to direct sunlight.

5. Wash the wells 3 times as in step 3.

6. Add **100 ul TMB substrate solution into all wells**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp. (20-25°C) 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 450/620nm** using an ELISA reader within 30 min after addition of the Stop Solution.

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^{O}C. Do not touch the bottom of the wells.