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

Catalog#	ProdDescription
920-100-AIV	Chicken Anti-Avian Influenza virus (AIV) IgG ELISA kit
920-010-PAG	Swine/Pig Anti-Influenza A virus IgG ELISA kit
920-020-PAM	Swine/Pig Anti-Influenza A virus IgM ELISA kit
920-030-PAA	Swine/Pig Anti-Influenza A virus IgA ELISA kit
920-040-HAG	Human Anti-Influenza A virus IgG ELISA kit
920-050-HAM	Human Anti-Influenza A virus IgM ELISA kit
920-050-HAM	Human Anti-Influenza A virus IgA ELISA kit
920-110-AIM	Chicken Anti-Avian Influenza virus (AIV) IgM ELISA kit
600-640-PMY	Swine/Pig Myoglobin ELISA Kit
6250-40	Swine/Pig Haptoglobin ELISA kit
80186	Swine/Pig Serum Antibody detection ELISA kit, Qualitative
9000	Swine/Pig Albumin ELISA Kit, 96 tests, Quantitative
9020	Swine/Pig IgG (total) ELISA Kit, 96 tests, Quantitative
9080	Swine/Pig IgM ELISA Kit, 96 tests, Quantitative
920-110-AV	Chicken Anti-Anemia Virus (AV) Ig's ELISA kit
920-120-NDV	Chicken Anti-Newcastle Disease Virus (NDV) Ig's ELISA kit
920-130-IBV	Chicken Anti-Infectious Bronchitis Virus (IBV) Ig's ELISA kit
920-140-MDV	Chicken Anti-Marek's Disease Virus (MDV) Ig's ELISA kit
910-100-JEM	Mouse Anti-Japanese encephalitis virus (JEV) Ig's ELISA kit
910-110-JWM	Mouse Anti-Japanese encephalitis virus (JEV) Ig's WB kit, 12 tests
900-100-83T	Mouse Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-120-83T	Rabbit Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-140-83T	G. pig Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-150-83T	Monkey Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-160-83T	Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit

Instruction Manual No. M-3300-420-HTM

Human Anti-Toxoplasma IgM

ELISA KIT Cat. # 3300-420-HTM, 96 Tests

For the detection of IgM antibody to Toxoplasma in human serum or plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Human Anti-Toxoplasma IgM ELISA KIT Cat. # 3300-420-HTM (96 tests)

Kit Components (96 tests)	Cat #
Toxoplasma antigen coated strip plate, (8x12 strip or 96 wells) # 3300421P	1 plate
Toxoplasma IgM **Negative Control** (1 mL) #3300422A	1 vial
Toxoplasma IgM **Cut-off Control** (1 mL) #93300422B	1 vial
Toxoplasma IgM **Positive control** (1 mL) #3300422C	1 vial
Enzyme Conjugate, (12 ml) # 3300423	1 bottle
Toxoplasma IgM **Sample Diluent**, 22 ml # 3300420SD	1 bottle
Wash buffer (20X), 25 ml # 3300420WB	1 bottle
TMB Substrate Solution, 12 ml # 3300420-TMB	1 bottle
Stop Solution, 12 ml # 93300420-ST	1 bottle
Complete Instruction Manual, M-3300-420-HTM	1

Intended Use

The Toxoplasma IgM ELISA kit is intended for the detection of IgM antibody to Toxoplasma in human serum or plasma. ***For research use only (RUO).***

Introduction:

Toxoplasma gondii is is an obligate intracellular parasite that infects about one third of the human population. It causes a zoonotic disease. Oocysts shed by final host (cats) can be introduced into humans by eating undercooked or raw meat, or drinking water contaminated with the oocysts. Infection of pregnant women may cause severe damage such as blindness, mental retardation, encephalitis, even though fetal death to her fetus via placental transmission of T. gondii, a proocess known as congenital toxoplasmosis. Accurate and rapid diagnosis of immune deficient individuals and prenatal diagnosis of pregnant women are important because of its capacity to induce severe clinical diseases such as encephalitis, ocular problems in retina with chorioretinitis, and congenital toxoplasmosis. ADI now offers the most sensitive, simple, and rapid ELISA kits for the detection of T.gondii antibodies in humans and animals (mouse, rat or g. pig etc). The ELISA kits are supplied with pre-coated plate sufficient for 96 tests, -ve and +ve controls and all required reagents. ADI T. gondii antibody ELISA produced results in 50 min at room temp and has a high sensitivity of ~ 1ng/ml T. gondii antibody in various samples

QUALITY CONTROL

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

1. The O.D. of the Calibrator should be greater than 0.250.

2. The Ab index for Negative control should be less that 0.9.

3. The Ab index for Positive control should be greater than 1.2

INTERPRETATION

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

Antibody Index Interpretation:

< 0.9 No detectable antibody to Toxoplasma IgM by ELISA

0.9 – 1.1 Borderline positive. Follow-up testing is recommended if necessary.

> 1.1 Detectable antibody to Toxoplasma IgM by ELISA.

References

1. Wilson M; Remington JS; Clavet C; Varney G; Press C; Ware D. Evaluation of six commercial kits for detection of human immunoglobulin M antibodies to Toxoplasma gondii.

2. Obwaller A; Hassl A; Picher O; Asp¨ock H. An enzyme-linked immunosorbent assay with whole trophozoites of Toxoplasma gondii from serum-free tissue culture for detection of specific antibodies. Parasitol Res 1995;81(5):361-4.

3. Loyola AM; Durighetto AF Jr; Silva DA; Mineo JR. Anti-Toxoplasma gondii immunoglobulins A and G in human saliva and serum. J Oral Pathol Med 1997; 26(4):187-91.

CALCULATION OF RESULTS:

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.

2. Calculate cut-off value: Calibrator OD x Calibrator Factor (CF).

3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Results:

Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

In order for an assay to be considered valid, the following criteria must be met If these criteria are not met, the test is not valid and must be repeated.

Results:

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

1. The O.D. of the Calibrator should be greater than 0.250.

2. The Ab index for Negative control should be less than 0.9.

3. The Ab index for Positive control should be greater than 1.2

PRINCIPLE OF THE TEST

Diluted serum is added to the wells coated with purified antigen. IgM 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 IgM specific antibody in the sample.). . Absorbance of is measured at A450 nm is using an ELISA plate reader.

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. 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.

MSDS

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

SPECIMEN COLLECTION AND HANDLING:

Use human serum or plasma (citrate) unknowns with this assay. If the assay is performed within 5 days after collection, the unknowns should be kept at 2-8°C; otherwise, they should be aliquoted and stored deep-frozen (70--20°C). If unknowns are stored frozen, mix thawed unknowns well before testing. *Avoid repeated freezing and thawing.* Heat inactivation of unknowns is not recommended.

REAGENTS PREPARATION

1. **Dilute Wash buffer 1:20 with water** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. **Store at room temperature (18-26 °C).**. 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^{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).*

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems, we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all unknowns and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
1 well	(e.g. B1)	for the negative control,
2 wells	(e.g. C1+D1)	for the cut-off control and
1 well	(e.g. E1)	for the positive control.

It is recommended to determine controls and unknowns in duplicate.
Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each control and unknown.
Adjust the incubator to 37° ± 1°C.

Assay Procedure:

1. Place the desired number of coated strips into the holder.

2. Negative control, positive control, and calibrator are ready to use. Prepare **1:21 dilution** of test samples, by adding **10 µl** of the sample to **200 µl** of sample diluent. Mix well.

3. Dispense **100 µl** of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense **100 µl** sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for **20 minutes at room temperature.**

4. Remove liquid from all wells. Wash wells three times with **300 µl of 1X wash** buffer. Blot on absorbance paper or paper towel.

5. Dispense **100 µl** of enzyme conjugate to each well and incubate for **20 minutes at room temperature.**

6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.

7. Dispense **100 µl** of TMB substrate and incubate for **10 minutes** at room temperature.

8. Add **100 µL** of stop solution.

9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

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.