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
920-060-HAA	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-4920

Human Anti-Taenia solium IgG (Cysticercosis) ELISA kit

Cat. # 4920, 96 Tests

For Qualitative Determination of IgG Antibodies to cysticercosis In Human Serum or plasma (citrate, heparin)

For In Vitro Research Use Only

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Human Anti-Taenia solium IgG (Cysticercosis) ELISA Cat. # 4920 (96 tests)

Kit Components (96 tests)	Cat #
Taenia solium antigen coated strip plate, (8x12 strip or 96 wells) # 4921P	1 plate
Taenia solium IgG **Calibrator A, Negative Control** (2 mL) #4922A	1 vial
Taenia solium IgG **Calibrator B Cut-off Standard** (3 mL) #4922B	1 vial
Taenia solium IgG **Calibrator C Positive control** (2 mL) #4922C	1 vial
Calibrators are ready-to-use, contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative	
HRP Conjugate, (20 ml) #4923	1 bottle
Sample Diluent, 100 ml #4920SD	1 bottle
Wash buffer (20X) 50 ml # 4920WB	1 bottle
TMB Substrate Solution, 15 ml # 4920TM	1 bottle
Stop Solution, 15 ml # 4920ST	1 bottle
Complete Instruction Manual, M-4920	1

Intended Use

ADI Human Anti-Taenia solium IgG (Cysticercosis) ELISA Kit has been designed for the detection of IgG class antibodies against Taenia solium in human serum or plasma (citrate, heparin). ***For In Vitro Research Use Only (RUO).***

Introduction

Cysticercosis the harboring of the larval form (Cysticerci) of Taenia, may involve any tissue of organ but are often associated with the central nervous system. Presence of the cysticerci in the brain may cause increased cranial pressure, seizure and altered mental states. Cysticercosis is acquired by ingestion of Taenia solium eggs and is rare in most developed countries but endemic in Latin America, Asia and Africa. Most cases of cysticercosis in the developed countries is associated with immigrants from developing countries (1).

Diagnosis of. cysticercosis usually requires multiple methods such as radiography and serology although use of cyst vesicular antigen has helped to increased its sensitivity and specificity, significant cross reactions with other diseases is still a problem. Specimens which test positive by a more specific test method such as immunoblot or other non-serological method (2-4).

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

Intraassay	n	Mean (E)	CV (%)
#1	24	0.460	4.89
#2	24	0.782	6.40
#3	24	0.790	8.29

Interassay	n	Mean (U)	CV (%)
#1	12	17.20	5.23
#2	12	20.32	7.84
#3	12	3.70	13.87

Clinical Sensitivity 100 %

Clinical Specificity 100% (95% confidence interval: 96.67% - 100%).

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.

Cross Reactivity:

Cross reaction of the antigens with antibodies against Echinococcus and Entamoeba is possible.

References

1. Flisser, A. and Larralde, C. Cysticercosis, Immunodiagnosis of Parasitic Diseases, Vol. 1, Ed. Walls and Schantz. Academic Press, pp.109-161 (1986).
2. Gottstein, et at. Species-specific Immunodiagnosis of Taenia solium cysticercosis by ELISA and immunoblotting. Trop Ned Parasi 38, 299-313 (1987).
3. Larralde, C. et al. Reliable serology of Taenia solium cysticercosis with antigens from cyst vesicular fluid: ELISA and hemagglutination tests. Am J Trop Med Hyg 35, 965-973 (1986).
4. Tsang, V. et al. An enzyme linked immuno-electro transfer blot assay & glycoprotein antigens for diagnosis of human cysticercosis (Taenia solium). J. Infect Dis. 159, 50-59 (1989).

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.

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 [U]

Sample (mean) absorbance value x 10 = [Units = U]

Cut-off

Example: 1.591 x 10 = 37 U (Units)

0.43

Interpretation of Results

Cut-off **10 U**

Negative **<9 U** The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

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**.

Positive **>11 U** Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).

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

PRINCIPLE OF THE TEST

Alpha Diagnostic's Taenia solium IgG (Cysticercosis IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Taenia solium antigens are 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 Taenia solium takes place. After an incubation step, the plate is rinsed with wash solution, in order to remove unbound material. Bound antibodies are detected with anti-human-IgG peroxidase conjugate. After a further washing step, the substrate (TMB) solution is added. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The color is measured using an ELISA reader at 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color. Results are obtained by comparing the A450 of the samples with the supplied negative and positive calibrators.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bi-distilled 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), Diluted H2SO4 (1N, stop solution), and Thimerosal (0.02% v/v in standards, conjugate diluent and HRP-conjugates).

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) must be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

REAGENTS PREPARATION

Dilute Wash buffer (20X) 1:19 with distilled water. (**e. g. 10 mL Washing Buffer + 190 mL distilled water.**) Store diluted buffer at 4ºC 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 6 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. **Calibrators are provided ready-to-use**. 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. Dilute the **wash buffer with distilled water (1:20).**

2. Dispense 100 µl diluent in 1 well to be used as blank. Pipet **100 µl of** ready-to-use calibrators, 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 µl Protein A 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 **15 minutes** at room temp. Blue color develops in positive controls and samples.

7. Stop the reaction by adding **100 µl 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^{o}C. Do not touch the bottom of the wells.