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

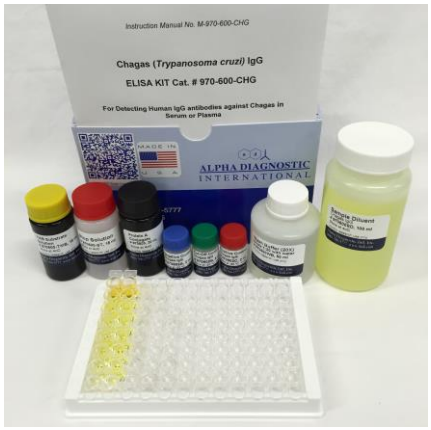
Catalog#	ProdDescription
920-100-AIV	Chicken Anti-Avian Influenza virus (AIV) IgG ELISA kit
920-010-PAG	Swine/Pig Anti-Influenza A virus IgG ELISA kit
920-020-PAM	Swine/Pig Anti-Influenza A virus IgM ELISA kit
920-030-PAA	Swine/Pig Anti-Influenza A virus IgA ELISA kit
920-040-HAG	Human Anti-Influenza 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-970-600-CHG

Chagas (*Trypanosoma cruzi)* IgG

ELISA KIT Cat. # 970-600-CHG, 96 Tests

For Detecting Human IgG antibodies against Chagas in Serum or Plasma



For In Vitro Research Use Only

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Email: Techsupport@4adi.com

Web Site: www.4adi.com

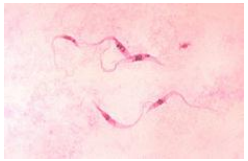
Human Chagas IgG ELISA KIT Cat. # 970-600-CHG (96 tests)

Kit Components (96 tests)	Cat #
Chagas antigen coated strip plate, (8x12 strip or 96 wells) # 970601P	1 plate
Chagas IgG **Negative Control** (2 mL) #970602A, blue cap	1 vial
Chagas IgG **Cut-off Control** (3 mL) #970602B, green cap	1 vial
Chagas IgG **Positive control** (2 mL) #970602C, red cap	1 vial
controls contains 0.1 % Kathon(see lot sp. conc on the vial)	
Protein A Conjugate, (20 ml) #970603	1 bottle
Chagas IgG **Sample Diluent**, 100 ml #970600SD	1 bottle
Wash buffer (20X) 50 ml # 970600WB	1 bottle
TMB Substrate Solution, 15 ml #970600-TMB	1 bottle
Stop Solution, 15 ml # 970600-ST	1 bottle
Resealable bag for the un-used antigen strips	1
Complete Instruction Manual, M-970-600-CHG	1

Intended Use

ADI Chagas (Trypanosoma cruzi) IgG-ELISA is intended for the determination of IgG class antibodies against Trypanosoma cruzi in human serum or plasma (citrate). This kit is for research use only, not for therapeutic uses.

Introduction:



The disease was first described in 1909 by **Carlos Chagas** after whom it is named**. Chagas disease or American trypanosomiasis**, is a tropical parasitic disease caused by the protozoan Trypanosoma cruzi. It is spread mostly by insects known as Triatominae or kissing bugs. The symptoms change over the course of the infection. In the early stage, symptoms are typically either not present or mild and may include: fever, swollen lymph nodes, headaches, or local swelling at the site of the bite. After 8–12 weeks, individuals enter the chronic phase of disease and in 60–70% it never produces further symptoms. The other 30 to 40% of people develop further symptoms 10 to 30 years after the initial infection. This includes enlargement of the ventricles of the heart in 20 to 30% leading to heart failure. An enlarged esophagus or an enlarged colon may also occur in 10% of people. The clinical manifestations of Chagas disease are due to cell death in the target tissues that occurs during the infective cycle, by sequentially inducing an inflammatory response, cellular lesions, and fibrosis. T. cruzi is commonly spread to humans and most mammals. The disease may also be spread through blood transfusion, organ transplantation, eating food contaminated with the parasites, and from a mother to her fetus.

Diagnosis of early disease is by finding the parasite in the blood using a microscope. Chronic disease is diagnosed by finding antibodies for T. cruzi in the blood. Various immunoassays for T. cruzi are available and can be used to distinguish among strains (zymodemes of T.cruzi with divergent pathogenicities). These tests include: detecting complement fixation, indirect hemagglutination, indirect fluorescence assays, radioimmunoassays, and ELISA. Alternatively, diagnosis and strain identification can be made using polymerase chain reaction (PCR).

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:

Inter-Assay-Precision:

	n	Mean (U)	CV %
Neg. Serum	4	5.7	10.2
Pos. Serum	7	13.5	7.7

Intra-Assay-Precision:

	n	Mean (U)	CV %
Pos. Serum	15	0.61	7.8

Specificity:

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 99 %.

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 0.2 mg/ml bilirubin.

References

http://en.wikipedia.org/wiki/Chagas_disease; "DPDx – Trypanosomiasis, American. Fact Sheet". Centers for Disease Control (CDC). Retrieved 12 May 2010; "Chagas disease (American trypanosomiasis) Fact sheet N°340". World Health Organization. March 2013. Retrieved 23 February 2014; Rassi A (2012) Infectious disease clinics of North America 26 (2): 275–91; Ben C (2007) JAMA 298, 2171-2181; El-Sayed NM, (2005). "The genome sequence of Trypanosoma cruzi, etiologic agent of Chagas disease". Science 309 (5733): 409–15; Dumonteil E, (2004). "Immunotherapy of Trypanosoma cruzi Infection with DNA Vaccines in Mice". Infect Immun 72 (1): 46–53.

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Results
A1, A2	Blanks	0.100	-
B1, B2	**Negative control**	0.210	0.110
C1, C2	**Cut-off control**	0.637	0.537
D1, D2	**Positive Control**	2.215	2.115
E1, E2	Sample 1	0.415	0.315
F1, F2	Sample 1	1.58	1.48

NOTE: These data are for **demonstration purpose only**. Use the values that are generated with each test.

Results:

1. **Run Validation Criteria:**

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** A450 value **< 0.100.**
- **Negative control** A450 value **< 0.200 and < cut-off**
- **Cut-off control** A450 value **0.150 – 1.30**
- **Positive control** **A450** value **> cut-off**.

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

Results:

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: A450 value Cut-off control 0.39 + absorbance value Cut-off control 0.37 =0.76 / 2 = 0.38

Cut-off = 0.38

Prevention mostly involves eliminating kissing bugs and avoiding their bites. Other preventative efforts include screening blood used for transfusions. A vaccine has not been developed as of 2013. Early infections are treatable with the medication benznidazole or nifurtimox.

PRINCIPLE OF THE TEST

T. cruzi IgG test kit is based on the principle of indirect ELISA. Microtiter strip wells are precoated with recombinant T. cruzi antigens to bind corresponding antibodies of the unknowns. After washing the wells to remove all unbound material horseradish peroxidase (HRP) labelled Protein A-conjugate is added. This conjugate binds to antigen-antibody complexes. The immune complex formed by the bound conjugate is visualized by adding TMB substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of T. cruzi-specific IgG antibodies in the unknowns. Stop solution is added to stop the reaction (converts blue color to yellow). . Absorbance of yellow color 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.

Sample Dilution

All samples should be diluted 1:100 with IgG Sample Diluent (Dispense 10µl samples in 1 ml IgG Sample Diluent gently mix).

REAGENTS PREPARATION

1. **Dilute Wash buffer 1:20 with 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 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. Dispense **100µl** controls and diluted unknowns into their respective wells. Leave well A1 for substrate blank. Cover wells with the foilt.

2. **Incubate for 1 hour ± 5 min at 37±1°C.**

3. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times **with 300µl of Washing Solution**. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step.

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

4. Dispense **100µl Protein A conjugate** into all wells except for the blank well (e.g. A1). Cover with foil.

5. **Incubate for 30 min at room temperature.** *Do not expose to direct sunlight.*

6. Wash as in step 4.

7. Dispense **100µl TMB Substrate** Solution into all wells. **Incubate for exactly 15 min at room temperature in the dark.**

8. Dispense **100µl Stop Solution** into all wells in the same order and at the same rate as for the TMB Substrate Solution. B*lue color changes to yellow. **Note**: Highly positive unknowns can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the unknown with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the unknown 1+100 with dilution buffer and multiply the results in U by 2.*

9. Measure the absorbance of the unknowns at 450/620nm 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.