T. Cruzi IgG ELISA Kit

Prod. No.: DEIA569
Pkg. Size: 96T

INTENDED USE

An enzyme immunoassay (ELISA) kit for the qualitative and semiqualitative detection of IgG antibodies anti-
Trypanosoma cruzi in human serum using recombinant anti-
gen.

GENERAL DESCRIPTION

Chagas disease or American Trypanosomiasis is an endemic and chronic disease. Its pathogenic agent is a flagellate protozoan called Trypanosoma cruzi which is transmitted to humans by a bug, triatominae. 3 stages can be present: acute, latent or indeterminate and chronic. The acute phase can present manifestations that include fever, myocarditis, lymphadenopathy, hepatosplenomegaly and parasitemia. Pseudocyst is formed after intracellular multiplication of parasite on muscles and on cells of endothelial reticulum system. Latent and intermediary stages are asymptomatic. The disease can evolve for the chronic phase with manifestations of myocardiopathy, degeneration of ganglion cells of peripheral and central nervous system and hypertrophy and dilatation of some organs such as oesophagus and colon. Chagas disease remains a major health problem in many Latin American countries because the high numbers of deaths. As the minority of patients presenting positive sorology for develop clinical evidence for chronic disease, information provided by the laboratory has a decisive and fundamental role for etiologic diagnostic. Chagas disease methods are: Complement fixation, agglutination, precipitation, immunofluorescence, hemagglutination and ELISA from which the most used are indirect hemagglutination (HAI), indirect immunofluorescence (IFI) and ELISA. ELISA methodology is increasingly used due to its easy-to-use format, excellent sensitivity and specificity and the possibility of automation.

PRINCIPLE OF THE TEST

The microtiter wells of the plate are covered with highly purified recombinant antigens of Trypanosoma cruzi. The specific antibodies Anti- Trypanosoma cruzi present in serum bind to these recombinant antigens. The unbound material is removed by washing and an antigammaglobulin anti-IgG human marked with peroxidase is added to the reaction. This conjugate binds to the specific antibodies anti- Trypanosoma cruzi IgG human bound on the antigen on the plate. The unbound material is removed again by washing and a substrate (TMB) is added which will develop a color on the wells in the presence of enzyme (peroxidase), indicating the presence of human antibody anti- Trypanosoma cruzi. The enzymatic reaction is stopped by the addition of a stop solution and the absorbance is measured at 450 nm. The concentration of specific IgG antibody is directly proportional to the intensity of the color of the reaction.

REAGENTS PROVIDED

1. Microplate with antigen of (12x8 wells).
2. Diluent solution lorange” (1 x 100ml)
3. Wash buffer - 20 x concentrated (1 x 50 ml)
4. Conjugate anti-human IgG Peroxidase (11ml)
5. Cromogen (TMB) (11ml)
6. Stop solution (11ml)
7. Negative serum control (2ml)
8. Positive serum control low (2,5ml)
9. Positive serum control high (2ml)
10. Instructions for use

STORAGE AND STABILITY

1. MICROPLATE: Stable if stored at 2-8°C up to the expiration date. Remove from the envelope the number of strips needed and let them reach to room temperature (20-25°C). Keep the remaining strips in the alluminium pouch with dessecant at 2-8°C.
2. DILUENT SOLUTION: Stable if stored at 2-8°C up to the expiration date. This solution is to dilute the specimens of the patient’s serum 1:25. After diluted, the specimens should be tested within 8 hours. Do not freeze. It contains Proclin 300-0.05% as preservative.
3. WASH BUFFER: Dulse wash buffer using 1 part of the buffer with 19 parts of distilled ou deionised water. During each washing cicle, each well should be filled with 300µl. Wash buffer is diluted and stable for 4 weeks at 2-8°C. It contains Proclin 300-0.05% as preservative. Allow the components to reach room temperature (20-25°C) prior to use.
4. CONJUGATE ANTI-HUMAN IgG PEROXIDASE: Ready for use. Stable if stored at 2-8°C up to the expiration date. Do not freeze. It contains Proclin 300-0.05% as preservative. Allow the components to reach room temperature (20-25°C) prior to use.
5. CROMOGEN (TMB): Ready for use. Stable if stored at 2-8°C up to expiration date. Do not freeze. It contains Proclin 300-0.05% as preservative. Allow the components to reach room temperature (20-25°C) prior to use.TMBis not mutagenic and carcinogenic.
6. STOP SOLUTION: Ready for use. This solution is hydrochloric acid (HCl), therefore, it is corrosive. Handle carefully. In case of skin contact, wash with abundant water. Allow the components to reach room temperature (20-25°C) prior to use. Stable up to the expiration date.

7. NEGATIVE SERUM CONTROL: Ready for use. Do not dilute. Store at 2-8°C up to expiration date. It contains Proclin 300 - 0.05% as preservative.

8. POSITIVE SERUM CONTROL LOW: Ready for use. Do not dilute. Store at 2-8°C up to the expiration date. It contains Proclin 300 - 0.05% as preservative. It must be used in duplicate to check the test performance and cut-off value.

9. POSITIVE SERUM CONTROL HIGH: Ready for use. Store at 2-8°C up to the expiration date. It contains Proclin 300 - 0.05% as preservative.

The kit presents good performance after being used for the first time. It is stable up to the expiration date if stored at 2 - 8°C.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes
2. Distilled or deionised water
3. Incubator at 37°C.
4. ELISA plate reader with 450nm filter
5. Paper towel.
6. Disposable material container

SPECIMEN COLLECTION AND HANDLING

Serum specimens free of haemolysis, lipaemia and contamination should be used. Plasma specimens with EDTA, heparin or oxalate can interfere on the results and should be avoided. Serum can be stored at 2-8°C for 48 hours. For longer storage, keep at -20°C up to 1 year. Frozen samples must be homogenised prior to test. Avoid air bubbles. Do not use Sodium azide as preservative since the enzyme peroxidase can be inhibited. After diluting the serum with diluent solution, use it within 8 hours. Avoid repeated freezing and thawing cycles.

ASSAY PROCEDURE

1. Reagent And Sample Preparation
   1) Dilute the serum to 1:25.
   2) Dispense 125µl of controls and diluted specimens at 1:25 in case of dilution in tube. With the fingers, beat on the plate edges or by mechanical vibration for 15 seconds for an homogeneous mixture.
   3) Cover the plate with an adhesive sheet, place the plate on a humid paper towel and incubate at 37°C for 60 minutes.
   4) Discard the material in a container with disinfectant solution.
   5) Wash the plate with diluted wash buffer. Fill and empty the well with 300µl of wash buffer. A manual or automatic microplate washer is recommended.

2. Assay Steps
   1) According to the established distribution plan, use 1 well for negative serum, 1 well for positive control high and 2 wells for positive control low, which will determine the cutoff value.
   2) Dispense 125µl of controls and diluted specimens at 1:25 in case of dilution in tube. With the fingers, beat on the plate edges or by mechanical vibration for 15 seconds for an homogeneous mixture.
   3) Cover the plate with an adhesive sheet, place the plate on a humid paper towel and incubate at 37°C for 60 minutes.
   4) Discard the material in a container with disinfectant solution.
   5) Wash the plate with diluted wash buffer. Fill and empty the well with 300µl of wash buffer. A manual or automatic microplate washer is recommended.

RESULTS

Determine OD for each test and serum control.

Cut off = OD Mean of positive serum control low

Validation of Test: OD Mean of positive serum control low should be 3 times higher than the OD of Negative Serum control.

INTERPRETATION

POSITIVE: OD of specimen is higher than borderline (dubious zone).

DUBIOUS RESULT (Borderline): OD of specimen is lower than O.D. of positive control serum low, but is higher than Cut-off value. It is called the dubious zone.

NEGATIVE: OD of specimen is lower than Cut-off value.
ASSAY CHARACTERISTICS

It was made tests to determine the sensitivity and specificity of Imuno-ELISA CHAGAS from CD. 120 known positive specimens were used and any false negative result was found. It awarded to Imuno-ELISA CHAGAS from CD a sensitivity of 100% on the specificity of the test. It was used 642 known negative specimens and any positive result was found what gave a specificity of 100% to Imuno-ELISA from CD.

REFERENCES