



# Chagatest

*ELISA*

Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *Trypanosoma cruzi*

## SUMMARY

Chagas' disease is a parasitic infection caused by *Trypanosoma cruzi*. The laboratory diagnosis depends on the stage of the disease. During the acute phase, the diagnosis is done by identification of parasite in blood or through immunological methods that detect IgM. During the chronic phase, immunological methods can be used, such as hemagglutination, immunofluorescence and enzyme immunoassay or western blot.

## PRINCIPLE

The sample is diluted in the well in which the antigen is immobilized. If sample contains specific antibodies, they will bind to the antigens and remain bound to the support. Unbound fraction is eliminated by washing. Then, the antibodies anti-human immunoglobulin conjugated to peroxidase is added which reacts specifically with immunocaptured anti-*T. cruzi* antibodies. The unbound conjugate is removed by washing. The presence of peroxidase bound to the complex is recognized by the addition of a chromogenic substrate. The reactive samples develop a light blue color. The enzymatic reaction is stopped by the addition of sulfuric acid, producing a light blue color change to yellow.

## PROVIDED REAGENTS

**Coated microtitration plate:** microtitration plate with removable strips with wells containing immobilized *Trypanosoma cruzi* cytoplasmatic and membrane antigens.

**Conjugate:** goat anti-human immunoglobulin conjugated to peroxidase.

**Substrate A:** 60 mmol/l hydrogen peroxide in 50 mmol/l citrate buffer, pH 3.2.

**Substrate B:** 0.01 mol/l tetramethylbenzidine (TMB) in 0.1 N hydrochloric acid.

**Stopper:** 2 N sulfuric acid.

**Concentrate Wash Buffer:** 1.4 mol/l sodium chloride in 100 mmol/l phosphate buffer and 0.1 g/l non-ionic surfactant.

**Sample Diluent:** bovine albumin in phosphate buffered saline (PBS), pH 7.2.

**Positive Control:** dilution of inactivated serum containing antibodies anti-*Trypanosoma cruzi*.

**Negative Control:** dilution of non-reactive inactivated serum.

## INSTRUCTIONS FOR USE

**Wash Buffer:** dilute 1 part Concentrated Wash Buffer + 4 parts distilled water. At low temperature, constituent reagents may precipitate. In that case, place at 37°C for a couple of minutes. Then mix by inversion.

**Coated microtitration plate:** ready to use.

**Conjugate:** ready to use.

**Substrate A:** ready to use.

**Substrate B:** ready to use.

**Stopper:** ready to use.

**Sample Diluent:** ready to use. The Sample Diluent color may vary from batch to batch without affecting its reactive capability.

**Negative and Positive Controls:** ready to use.

## WARNINGS

- Reagents are for "in vitro" diagnostic use.
- All samples should be handled as capable of transmitting infection. Control sera are inactivated, however, they should be handled as infectious material.
- Control sera have been tested and found non-reactive to Hepatitis B Surface Antigen (HBsAg), antibodies to Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV).
- All materials used to perform the test must be destroyed to ensure the inactivation of pathogenic agents. The recommended method is autoclaving for 1 hour at 121°C. Liquid waste may be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.
- Do not exchange reagents from different kits and lots.
- Do not use reagents from other origin.
- Microtitration plates must be placed in incubator. Do not use water bath. Do not open the incubator during the process.
- Avoid the contact of microtitration plates with hypochlorite fumes from biohazard disposal containers or other sources, as hypochlorite affects the reaction.
- Avoid contact of the sulfuric acid (Stopper) with the skin and eyes. If this occurs, rinse immediately with plenty of water.
- Avoid the spill of liquids and the formation of sprays.

## STABILITY AND STORAGE INSTRUCTIONS

**Provided Reagents:** stable at 2-10°C until the expiration date stated on the box. Do not freeze.

**Wash Buffer:** stable for up 3 months at room temperature.

**Coated microtitration plate:** well strips with immobilized antigen are provided in vacuum-sealed pouches with desiccant. The pouch must be brought to room temperature before opening. Besides, do not open it until performing the test. Otherwise, the well strip surface could get moistened. Unused well strips should be kept in the pouch with desiccant, sealed and stored at 2-10°C. Test strips stored in this manner are stable for 4 months if it does not exceed the expiry date stated on the box label.

## SAMPLE

Serum or plasma

**a) Collection:** obtain the sample in the usual way. Do not use heat-inactivated samples.

**b) Additives:** not required for serum. Employ plasma collected in EDTA, heparin or citrate base anticoagulants.

**c) Known interfering substances:** hemolysis, hyperlipemia or other causes of turbidity may yield erroneous results. Clarify this samples by centrifugation.

**d) Stability and storage instructions:** undiluted samples can be stored for up to 7 days at 2-10°C. If they have to be stored for longer periods, they should be frozen at -20°C or less.

Samples should not be repeatedly frozen and thawed. This may lead to erroneous results.

If samples are to be transported, they could be packaged according to local regulations for biohazard material shipment.

## REQUIRED MATERIAL (non-provided)

- Micropipettes for measuring the stated volumes.
- Stopwatch.
- 37°C incubator.
- Spectrophotometer for microtitration plates reading (optional).

## ASSAY CONDITIONS

- Primary wavelength: 450 nm
- Secondary wavelength (bichromatic): 620-650 nm
- Instrument calibration: set the spectrophotometer to zero with the Reagent Blank.
- Total reaction time: 90 minutes
- Temperature reaction: 37°C and room temperature
- Sample volume: 10 ul

## PROCEDURE

Bring the reagents and the samples to room temperature before starting the test. Once the test has begun, it should be performed without interruptions.

In addition to the specimens (Unknown-U) 2 Positive Controls (PC) and 3 Negative Controls (NC) must be included. When placing the sample and/or Controls on the Sample Diluent, make sure that they are placed on the center of the liquid, and not on the walls or bottom of the well. Dispense the Sample Diluent. Homogenize by loading and unloading the micropipette.

In the microtitration plate wells to be used, place:

	U	PC	NC
<b>Sample Diluent</b>	200 ul	200 ul	200 ul
<b>Positive Control</b>	-	10 ul	-
<b>Negative Control</b>	-	-	10 ul
<b>Sample</b>	10 ul	-	-

Once the samples have been dispensed on each strip, mix by gently tapping the sides of the microtitration plate for 10 seconds.

To avoid evaporation, cover the microplate with a plate

sealer and placed 30 minutes at 37°C. Then, carefully aspirate the fluid from each well into a biohazardous container with 5% sodium hypochlorite. Then, wash 5 times with Wash Buffer, using approximately 300 ul/time/well. After each wash, the liquid should be discarded into the container with hypochlorite. An automated washer can be use.

After the last wash, invert the plate and firmly tap on a clean paper towel to remove excess wash buffer (gently pressing with the hand the long sides to prevent the well strips from falling). Then add to each well:

<b>Conjugate</b>	1 drop	1 drop	1 drop
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When using an automated micropipette, dispense 60 ul. Mix by gently tapping the sides of the microtitration plate for 10 seconds.

To avoid evaporation, cover the microplate with a plate sealer and placed 30 minutes at 37°C. Then, carefully aspirate the fluid from each well into the container with hypochlorite, and wash as indicated above.

After the last wash, invert the plate and firmly tap on a clean paper towel to remove excess wash buffer (gently pressing with the hand the long sides to prevent the well strips from falling).

Then, add to each well:

<b>Substrate A</b>	1 drop	1 drop	1 drop
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<b>Substrate B</b>	1 drop	1 drop	1 drop
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When using an automated micropipette, dispense 50 ul. Mix by gently tapping the sides of the microtitration plate for 10 seconds. Incubate 30 minutes at room temperature. Then add:

<b>Stopper</b>	1 drop	1 drop	1 drop
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When using an automated micropipette, dispense 50 ul. Mix by gently tapping the sides of the microtitration plate for 10 seconds.

Measure optical density at 450 nm or bichromatic at 450/620-650 nm, or evaluate the result with the naked eye by comparing it with the Positive and Negative Controls.

## STABILITY OF FINAL REACTION

Reaction color is stable for 30 minutes. Thus, results should be read within this period.

## ASSAY VALIDATION CRITERIA

The assay is considered valid if the following conditions are simultaneously met:

a) Readings of at least 2 out of the 3 Negative Controls, corrected against the Reagents Blank, should be lower than or equal to 0.150 O.D.

b) The mean optical density of the Positive Controls should be less than or equal to 0.600 O.D.

In case one of the above conditions is not met, repeat the assay. Remember that the obtained readings will depend on the sensitivity of the instrument used.

## INTERPRETATION OF RESULTS

### a) With optical instruments:

The presence or absence of antibodies anti-*T. cruzi* is determined associating the sample absorbance with the Cut-off value.

Cut-off = NC + 0.200 O.D.

NC: O.D.mean of the Negative Control

Indetermination area: Cut-off  $\pm$  10%

**Non reactive samples:** samples with absorbances lower than the Cut-off value.

**Reactive samples:** samples with absorbances greater or equal to the Cut-off value

**Indeterminate Samples:** if sample absorbance fall within the indetermination area, the assay should be repeated.

### b) Visual interpretation:

If this type of interpretation is selected, every sample not presenting higher intensity coloration than the Negative Control should be considered non-reactive.

Otherwise, an evidently yellow sample is considered Reactive. Color intensity higher than the Negative Control, require instrumental interpretation.

## PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

- The following causes lead to erroneous results:

Improper washing of the wells.

Cross contamination of non-reactive samples with antibodies from a reactive sample.

Chromogenic solution contamination with oxidizing agents (chlorine, etc.).

Stopper contamination.

Improper storage of unused strips wells.

Diluted Wash Buffer contamination. Verify that clean bottles were used to prepare and store the diluted wash buffer. If turbidity or precipitation are observed, solution should be discarded.

- A negative result does not exclude the possibility of exposure or infection by *T. cruzi*.

- Occasionally negative absorbance may be obtained when performing bichromatic readings that do not invalidate the determination. This is owed to some samples that yield readings below the Reagent Blank.

- Do not use samples heat inactivated, may lead to false positive results.

- Check that the washing system works properly.

## PERFORMANCE

**a) Sensitivity:** in a study performed on a panel of 94 samples with positive serology by two coincident references methods (indirect hemagglutination and indirect immunofluorescence), the sensitivity was 100%.

**b) Specificity:** in a study performed on a panel of 348 samples with negative serology by two coincident references methods (indirect hemagglutination and indirect immunofluorescence), the specificity was 99.6%.

**c) Population-based studies:** in a population including individuals with different clinical conditions, the correlation with other reference methods was 99.7%.

It is recommended that any Reactive result be investigated by further diagnostic testing. The «Fatala Chabén» Institute recommends criteria by which the immunodiagnostic of the infection should be performed with a minimum of 2 of the following methods, validated by the Reference National Center: indirect immunofluorescence, indirect hemagglutination and EIA.

## WIENER LAB. PROVIDES

Kit for 96 tests (Cat. 1293251)

Kit for 192 tests (Cat. 1293252)

## REFERENCES

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