

CELQUEST CHAGAS ELISA

ELISA recombinant

Immunoenzymatic assay for the detection of IgG antibodies to *Trypanosoma cruzi*.

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Only for in vitro diagnosis. Immunoenzymatic assay for the qualitative detection of IgG antibodies to *Trypanosoma cruzi* in blood or plasma.

I) CLINICAL SIGNIFICANCE

Chagas is a chronic disease caused by the infection with the protozoan *T. cruzi*. The parasite is transmitted to humans by a group of insects of the Roduvidae family, being the *Triatoma infestans* the main vector. The infection can also be transmitted congenitally, by blood transfusion or organ transplant. This infection affects diverse organs and systems, specially the heart and the digestive tube. The illness is endemic from Southern USA to central Chile and the South of Argentina.

The immune-humoral response to the infection can be rapidly detected by CELQUEST CHAGAS ELISA. This assay is appropriate for the analysis of a vast number of serum or plasma samples, thus being especially useful in blood banks and clinical laboratories.

II) PRINCIPLE OF THE TEST

CELQUEST CHAGAS ELISA is a solid phase immunoenzymatic assay for qualitative detection of antibodies to *T. cruzi*. It is performed on microplates which are sensitized with *T. cruzi* recombinant antigens, designed and developed in our laboratory. If the samples analyzed contain specific antibodies to *T. cruzi*, these will form a stabilized complex with the antigens that coat the wells. Non specific bonds will be eliminated by washing. During the incubation with the conjugate, the anti-IgG antibodies marked with peroxidase will join the formed complex. Finally, in the incubation phase with the chromogenic substrate, the peroxidase bonded to the complex will produce a coloration, which will allow the detection of the reactive samples to *T. cruzi*. After stop the enzymatic reaction by the addition of sulfuric acid, the color intensity should be measured with a colorimetric reader for ELISA microplates.

1) COMPONENTS

CELQUEST CHAGAS ELISA, components for 96 determinations:

- An ELISA microplate for 96 determinations, sensitized with recombinant *T. cruzi* antigens. Each microplate is made up by 12 strips of 8 wells that can be used individually and comes inside a hermetically sealed package containing silica gel.
- Self adhesive seals for sealing the microplates.
- Bag for preserve remaining microplate strips
- The solutions contained in the kit are detailed in Table 1.

Table 1

Solution	Description
Washing solution (concentrate 25x)	60ml bottle containing buffer solution, pH 7.2. Contains Tween 20.
Sample diluting solution	30ml bottle containing buffer solution, pH 7.2. Contains Tween 20, BSA and aditives
Anti-IgG conjugate	15ml bottle containing Anti-IgG conjugate solution (goat) marked with peroxidase. Contains stabilizers.
Substrate	15ml bottle containing Tetrametilbenzidine (TMB) and hydrogen peroxide. This is a very sensitive reactive and must not be used if it shows shadiness or a blue precipitation.
Positive control	500 µl tube containing chagasic human serum.
Negative control	500 µl tube containing human serum which is negative for Chagas.
Sulfuric acid (H2SO4) 2N	10ml of the solution. In case of contact with skin or mucous membrane, profusely wash the affected zone with water.

III) MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes or multi-channel pipettes.
- Dispensable tips for micropipettes
- Distilled or deionized water
- Microplate automatic washing system (optional)
- Microplate reader with a 450nm filter and a reference filter (620 or 630 nm)
- 37°C incubator

IV) WARNINGS AND PRECAUTIONS

- Use only for in vitro diagnosis
- The human sera used as controls have shown negative results for HTLV, Syphilis, HIV, HBV and HCV, however it is recommended to handle them as potential infectious samples. At present any known assay warrants the absence of infectious agents in blood derivatives.
- All the reagents and components of this kit must reach room temperature before being used and must be refrigerated at 2-8°C after usage.
- Do not open the microplate package until the it has reached room temperature. Store the non-used strips along with the silica gel, inside the provided plastic container. The container must remain well closed.
- Those recipients used for the preparation of the reagents must be clean and detergent and chlorine free.
- Use new tips for every sample and reagent.
- Avoid touching the well with the tip.
- Never pipette directly from the original bottle.
- Never return any rest of reagent to the original bottles.
- Do not use reagents from different batches.
- The reproducibility of the test depends on the pipetting precision, the precision of the incubation timing and temperature and the correct washing of the wells.

- Do not allow any metallic object to contact the solutions.
- Do not use contaminated samples or reagents, since they may produce false results.

V) **SAFETY PRECAUTIONS**

- Use gloves during the manipulation of samples and reagents.
- Do not use the mouth for pipetting.
- Do not eat, drink, smoke or manipulate contact lenses in the working-area.
- Clean and disinfect any spill of the samples or reagents using 0.5% Sodium Hypochlorite.
- Avoid contact of the stop solution with the skin or mucouses. If this or any other reagent contacts the skin or mucouses, wash the affected area with abundant water.
- Eliminate all contaminated material using wasting recipients which are suitable for biological waste. This recipient may be decontaminated using an autoclave at 121°C for one hour or by treatment with 0.5% Sodium Hypochlorite for two hours.
- The sample remains and used controls and reagents, must be treated with 0.5% Sodium Hypochlorite for two hours before its elimination.

VI) **STORAGE AND TRANSPORTATION CONDITIONS**

- CELQUEST CHAGAS ELISA must be stored and transported at 2-8° C. Do not freeze. The stability tests demonstrate that CELQUEST CHAGAS ELISA preserves its initial activity after one week at 37° C. Yet, it is recommended to follow the storage conditions described above.

VII) **PREPARATION AND STORAGE OF THE SAMPLES**

- CELQUEST CHAGAS ELISA can be used both with serum and human plasma.
- Anticoagulants as EDTA, oxalate, heparin or citrate, that may be present in the samples, do not affect the test results.
- Samples containing precipitates or clots must be centrifuged at 2500 rpm for 10 minutes before the test. **Warning:** do not use samples with microbial contamination since they may lead to false results. Samples may be stored at 2-8° C up to one week before testing. For longer periods add 0.1% sodium azide and freeze at -20° C or a lower temperature. Do not perform repeated freeze-thaw cycles with the samples, since this may affect its stability. **Warning:** do not freeze the samples in freezers with auto-defrost systems. Frozen samples must be homogenized and centrifuged before using.

VIII) **PROCEDURE**

Wait until the reagents reach room temperature, before starting the assay.

1. Dilute the 25X wash buffer concentrate (i.e. To prepare 500mL Washing solution add 20mL of washing buffer to 480mL of water). If 25X solution presents crystals, dissolve them by shaking gently before use.
2. Place 200 µL of sample diluent in each well to be used. Consider two wells for the blank reaction, two wells for the positive control and two wells for the negative control.
3. Add 10 µL of serum or plasma to each well, including controls.
4. Seal the microplate with the adhesive seal included in the kit, in order to avoid the evaporation of the reagents. Incubate for 30 minutes at 37° C +/- 1.
5. Wash the microplate using 350 µL of diluted washing buffer (1X) per well for 30 seconds each time and discard the buffer after each wash. It is recommended to wash 5 times with an automated microplate washer or by manual washing.

Recommended protocol for automated washing of stripes:

Perform five washing cycles dispensing 350 µL of diluted washing buffer, making sure that:

- a. The diluted washing buffer fills the well.
- b. The diluted washing buffer does not spill from the well.
- c. The diluted washing buffer remains 30 seconds inside the well.
- d. There is no remaining liquid inside the well after finishing washing.

Notice: always maintain the equipment clean by rinsing with abundant distilled water at the end of each working journey. Perform periodical maintenances according to the manufacturer's recommendations.

6. After the last wash invert the microplate over an absorbent paper and tap. **Do not allow the microplate to dry.**
7. Add 100 µL of conjugate in all the wells.
8. Seal the microplate in the same way than before the previous incubation and incubate 30 minutes at 37° C +/-1. Wash the microplate using the Washing Buffer just as in step 5 and 6.
9. Developing: add 100 µL of substrate to each well. Incubate the microplate **exactly 30 minutes** at room temperature.
10. Interrupt the reaction by adding 50 µL of stop solution to each well.
11. Measure optical density at 450nm or bichromatic at 450-620 to 650

IX) **READING AND INTERPRETATION OF THE TEST RESULTS**

Perform the reading of the microplate as soon as possible.

Warning: readings performed after 60 minutes are not reliable.

Quality Control

Assay's results are valid if they comply the following criteria:

Mean absorbance for blanks: lower than 0.15

Negative control: absorbance lower than 0.20 after subtract blank value

Positive control: absorbance equal or higher than 1.0.

Results

1. Calculate the mean absorbance of the negative control and add 0,4. The obtained value is the threshold value.
2. Divide the sample absorbance by the threshold value.
 - Positive: absorbance/threshold value ≥ 1.1
 - Negative: absorbance/threshold value < 0.9
 - Uncertain: absorbance/threshold value $\geq 0.9 < 1.1$

Interpretation of the results

A positive result indicates the presence of antibodies IgG anti- *T. cruzi*.

A negative result indicates the absence of detectable levels of antibodies anti- *T. cruzi*. This may be due to the absence of infection or the patient's weak immune response.

If the result is uncertain, repeat the assay in duplicate. If the reading persists uncertain consider it as a positive result.

X) **LIMITATIONS OF THE ASSAY**

- All the uncertain or positive samples must be repeated in duplicate. The samples that are repeatedly positive may be confirmed by techniques such as: IFI, Western Blot, Xeno-diagnostic or PCR.
- The diagnosis of Chagas Disease must be based in a combination of results including the patient's clinical history, the detection of the parasite and future serological assays.
- Microbial contamination of the samples, patients with other parasitosis or self-immune diseases may produce false positive results.
- A negative result does not exclude the possibility of infection with *T.cruzi*. An immune response can not be detected during the first weeks after infection by any available method.

XI) PERFORMANCE CHARACTERISTICS

CELQUEST CHAGAS ELISA has been tested using plasma and sera from diverse origins and has been compared to other commercial assays.

- Sensitivity and Specificity study: a ROC curve was built using a panel containing 881 samples, establishing a sensitivity of 99.5% and a specificity of 100%.
- Comparative study: CELQUEST CHAGAS ELISA was compared to 2 commercial kits using a panel of 207 samples. The results are shown in Table 2.

Table 2: Comparative study results.

	Assay					
	CelQuest		Assay A		Assay B	
	Negative	Positive	Negative	Positive	Negative	Positive
Results	74/74	133/133	74/74	129/133	73/74	132/133
Concordance	100%		98.1%		99.0%	

Assay A is made from parasite extracts and detects IgG.

Assay B is made from recombinant antigens and detects IgG.

XIII) REFERENCES

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