TRYPANOSOMA CRUZI qPCR TEST KIT HANDBOOK

CAT NO.: YSL-qP-IC-T.cruzi-100

100 reactions
with Internal Control and Lyophilised MasterMix

VERSION 7.3

For research use only

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INTENDED USE

This product is a qPCR test kit for detection and quantification of Trypanosoma cruzi (T.cruzi) DNA in good quality nucleic acid samples from a variety of sources. It is designed to be used by trained users in a suitable molecular biology laboratory environment.

KIT CONTENTS

	Cap Colour	Volume
T.cruzi specific primer/probe (FAM Probe)		100 µl
Internal extraction control primer/probe (VIC/HEX Probe)		100 µl
DNase/RNase free water		1.5 ml
Template Resuspension Buffer (TRB)		1.5 ml
ROX passive reference dye		10 µl
Lyophilised Tetra™ 2X qPCR MasterMix		1.1 ml*
MasterMix Resuspension Buffer (MMRB)		1.5 ml
T.cruzi Positive control template		500 µl*
Internal extraction control DNA template		500 µl*

* Supplied lyophilised and requires resuspension before use, see resuspension step below for instructions

RESUSPENSION

Resuspend the designated kit contents with the correct reagents as per the table below. Spin or gently tap the vial/tube to ensure all contents are at the bottom before opening.

After adding the resuspension reagent, pulse vortex the vial/tube to ensure each is mixed well.

	Reagent	Volume
Lyophilised Tetra™ 2X qPCR MasterMix	MMRB	1.1 ml
T.cruzi Positive control template	TRB	500 µl
Internal extraction control DNA template	TRB	500 µl

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ROX (PLATFORM DEPENDENT)

ROX is required for platforms that use a passive reference dye. The table below outlines the platforms that require the addition of ROX in the MasterMix.

If ROX is required, dilute the ROX supplied according to the table below, then add 5µl to the fully resuspended Tetra™ MasterMix.

	Instruments	Step 1: Volume of water to add to ROX tube	Step 2: Add to MasterMix vial
High ROX Instruments:	Applied Biosystems 7700, 7000, 7900, 7300, StepOne, StepOne Plus, and Roche capillary Lightcyclers 2.0	No Dilution Required	5 µl
Mid ROX Instruments:	Stratagene MX	75 µl	5 µl
Low ROX Instruments:	Applied Biosystems 7500 Platform, ViiA7 platforms, Quantstudio	130 µl	5 µl
ROX Not Required:	All Other Machines	Not Required	Not Required

MATERIALS REQUIRED BUT NOT PROVIDED

DNA Extraction kit – This qPCR test kit will work well with high quality DNA derived from any extraction kit with minimal PCR inhibitors present.

qPCR instrument with minimum 2 colour detection (FAM and VIC/HEX).

Pipettes, micro centrifuge tubes and general laboratory equipment.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection and quantification of Trypanosoma cruzi is designed to have the broadest detection profile possible and detect all clinically relevant strains. The primers and probes have very high (>95%) homology with all reference data within the NCBI database.

The target gene for Trypanosoma cruzi (kDNA) has been demonstrated to have a distinctive sequence making it an ideal target for highly specific detection of the target.

The primers will detect Trypanosoma cruzi and the following strains: Trypanosoma cruzi marinkellei, Trypanosoma cruzi strain CL Brener, Trypanosoma cruzi strain Esmeraldo, Trypanosoma cruzi JR cl. 4, Trypanosoma cruzi Tula cl2.
However due to extreme homology it is possible that the kit may also detect some species of Trypanosoma rangeli and Trypanosoma copemani.

If you require more specific data about the detection profile of the kit, please do not hesitate to contact our bioinformatics team : support@youseq.com

USE OF DNA INTERNAL CONTROL

Add 5 µl of the internal extraction control DNA into the extraction/lysis buffer for each sample that you are extracting. Make sure not to add this DNA directly in to your biological sample as this may cause degradation of the control DNA.

Continue DNA extraction as per the manufacturer's method.

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

In a relevant sized sterile container (e.g., Microcentrifuge tube or Falcon tube), combine the following reagents to create a Reaction Mix to cover the number of reactions you are running. Mix the combined reagents by briefly vortexing or inverting.

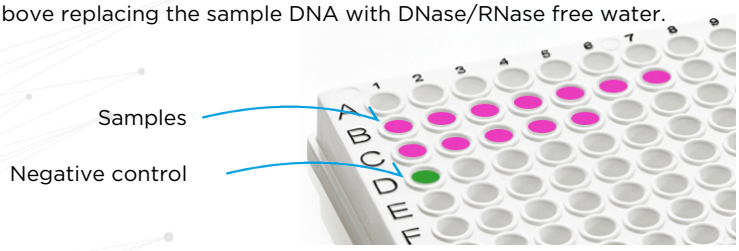
Component	Volume	Volume x N
Tetra™ 2X qPCR MasterMix	10 µl	10 x N
T.cruzi specific primer/probe	1 µl	1 x N
Internal extraction control primer/probe	1 µl	1 x N
Reaction Mix Volume	12 µl	X µl

Then dispense 12µl of the reaction mix into all the required wells and add 8µl of sample into the relevant wells.

Component	Volume
Reaction Mix (primer/probe & MasterMix)	12 µl
Extracted Sample DNA	8 µl
Final Volume	20 µl

NEGATIVE CONTROL

For a negative control, repeat the reaction set up above replacing the sample DNA with DNase/RNase free water.



Please note: We advise sealing the sample and negative control wells before proceeding to the post-PCR environment (positive control step).

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POSITIVE CONTROL

In your designated post-PCR environment, perform a serial dilution of the Positive control template to create a six-point standard curve.

1. Add 90 µl of TRB into 5 tubes and label them 2, 3, 4, 5 and 6.
2. Pipette 10 µl of Positive control template into tube 2
3. Mix by pipetting up and down 5 times
4. Change pipette tip and pipette 10µl from tube 2 into tube 3
5. Mix by pipetting up and down 5 times

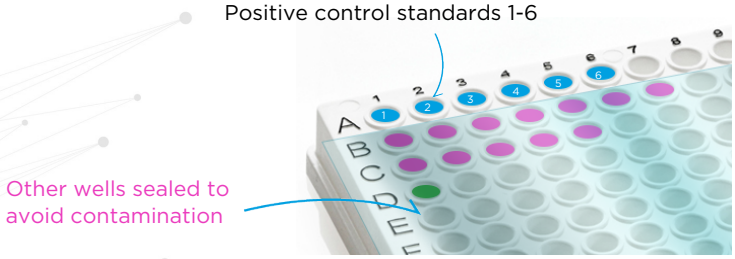
Repeat steps 4 and 5 for the remaining tubes to complete the dilution process.

Combine the following reagents for each point of the standard curve:

Component	Volume
Tetra™ 2X qPCR MasterMix	10 µl
T.cruzi specific primer/probe	1 µl
Internal extraction control primer/probe	1 µl
DNase/RNase free water	3 µl
Designated dilution of Positive control template	5 µl
Final Volume	20 µl

The described standard curve provides a dynamic range as in the table below:

Tube No.	Copies of Target / rxn
1	1,000,000
2	100,000
3	10,000
4	1,000
5	100
6	10



qPCR AMPLIFICATION PROTOCOL

This YouSeq kit will work with any qPCR instrument capable of detecting FAM and VIC/HEX.

Run the following qPCR protocol:

	Temperature	Time
Hot Start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

*Make sure to collect fluorogenic data through both the FAM and VIC/HEX channels during this step

INTERPRETATION OF RESULTS

When analysing Sample Cq values, YouSeq recommends checking the threshold within the run file before interpreting the data. We would suggest setting the threshold to 10% of the relevant positive control End Point Fluorescence (EPF).

Positive control

Firstly, check the positive control performance. The undiluted positive control should amplify in a Cq range of 18.5 ±2. If the Cq range is not achieved, this would be a failed test and should be repeated.

If running a positive control standard curve for a quantitative result, then an efficiency between 90% to 110% will provide the most accurate quantification. This will be automatically calculated by your analysis software. If it falls outside of this range, then preparing a fresh standard curve and repeating the run may improve the efficiency value.

Please note: The supplied positive control is a sequence representative of the target region and does not contain the organisms entire genome.
The positive control does not include the control sequence and therefore, should not be expected to amplify in the VIC/HEX channel.

Negative control

In ideal circumstances, the negative control should deliver a flat line – negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal. If the negative control signal is ≥5 Cq values later than your sample signal, then it can be considered negative and the sample result is valid.
If the negative control is <5 Cq later than the sample, the result is invalid and the test should be repeated after potential sources of contamination have been removed.

The test is valid if the following conditions are met:

	FAM Channel
Positive Control (Tube 1)	+
Negative Control	-

INTERPRETATION OF RESULTS CONTINUED

Positive samples

Samples that are positive for Trypanosoma cruzi will deliver a defined "sigmoidal" amplification plot. Your qPCR instrument software will calculate a quantitative result for these signals by comparing the signal to the positive control standard curve. Results will be presented as target copy number.

Internal extraction control

Detection of the internal extraction control is through the VIC/HEX channel. This control gives information about the efficiency of your DNA extraction step. Cq values in the range 28±3 indicate a good quality extraction has taken place. If your signal is later than this, you may wish to consider repeating the DNA extraction.

Results interpretation at a glance:

	qPCR Signal		
T.cruzi (FAM)	≤35	-	-
Internal extraction control (VIC/HEX)	+/-	+	-
Positive control (FAM)	+	+	+
Negative control	-	-	-
Result	Positive result	Negative result	Failed test. Insufficient extraction.

PRODUCT SPECIFICATIONS

How sensitive is my kit?

The kit is suitable for the detection of Trypanosoma cruzi, across a wide dynamic range. Under ideal PCR conditions the kit can detect less than 100 copies of the target in the PCR reaction.

Storing your kit

Store at -20°C from arrival. The qPCR kits shelf life is outlined as an expiry date on the pouch label.
Once you have prepared the positive control standard curve it can be stored frozen. However, if you observe a shift in Cq values in the standard curve over time a fresh standard curve should be prepared.

Use good quality DNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality DNA. Good quality is defined as DNA with high integrity (not degraded) and with low levels of inhibitors present.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of Trypanosoma cruzi qPCR Test Kit is tested against predetermined specifications to ensure consistent product quality. The design of the kit met our robust bioinformatic analysis requirements resulting in a clinically relevant detection profile based on available sequence information. The kit is periodically checked against newly available sequence information to remain clinically relevant.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
phone: +44 (0)333 577 6697

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