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Blood DNA extraction protocol compatible with LAMP-PCR for T. cruzi detection.

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This cost-effective protocol uses CTAB lysis buffer and isopropanol precipitation to purify high-quality nucleic acids from fresh peripheral blood collected in EDTA. The protocol is about four times cheaper when compared with commercial kits. The isolated DNA is compatible with both LAMP-PCR and qPCR assays, providing reliable results for downstream molecular applications.

Guidelines

Compared to commercial kits, this protocol yields a comparable average DNA amount but with moderately higher variability.

Materials

Reagents

	A	B	C
	Reagent	**Company**	**Catalog number/SKU**
	2-propanol	sigma aldhrich	1096341011
	Hexadecyltrimethylammonium bromide	sigma aldhrich	H5882-100G
	Tris-HCl, 1M Solution, pH 8.0, Molecular Biology Grade, Ultrapure	Fisher scienific	AAJ22638AE
	Ethylenediaminetetraacetic acid solution	MERK	E8008-100ML
	Absolute ethanol	Thermo scientific	T038181000
	Nuclease free water	Milipore	4.86505.1000
	NaCl (5 M), RNase-free	Milipore	S9625-1Kg

Disposable materials

	A	B	C
	Disposable material	**Company**	**Catalog number/SKU**
	Conical Base 2.0 mL Microcentrifuge Tubes	USA Scientific	14202700
	1000 uL tips (TipOne‰ Pipette Tips in Racks)	USA Scientific	1111-0806
	200 uL tips (TipOne‰ Pipette Tips in Racks)	USA Scientific	1111-0806

Permanent materials

	A	B
	Permanent materials/equipment	**SKU**

	A	B
	Vortex mixer	CLS6885DB
	Centrifuge (capable of 12,000 × g) (Benchmark MC-12 High Speed Microcentrifuge)	BMS:C1612
	Heater (prewarmed to 70 °C) (Corning‰ LSE‰ digital dry bath heater)	CLS6885DB
	Plastic Test Tube Rack Cube	76263-262

Safety warnings

It is recommended to receive training before processing important samples and to perform extractions in replicates when possible.

Before start

Collect peripheral blood in an EDTA vacutainer and proceed with the checklist section.

Check List (Ensure you have the following materials ready before running the experiment)

10m 30s

1 **Equipment**· 30s

- Vortex mixer
- Centrifuge *(capable of 12,000 × g)*
- Heater *(prewarmed to 70 °C)*
- Timer

2 **Cold Storage**· 2m

- A bucket with ice

3 **Reagents & Buffers** 30s

- CTAB 2% lysis buffer *(CTAB: 2% (w/v), Tris-HCl (pH 8.0): 100 mM, EDTA: 20 mM, NaCl: 1.4 M.*
- 5 M NaCl *(cold/onice)*
- Isopropanol *(cold/onice)*
- 70% ethanol
- PCR-grade nuclease-free water *(incubate at 70 °C)*

4 **Consumables & Labware**· 2m

- Rack for 2 mL tubes
- 2 mL microcentrifuge tubes *(Catalog 1420-2700 USA Scientific; two per sample)* Do not use low-binding tubes.
- 1 mL pipette + tips
- 100 µL pipette + tips
- Paper towel
- Dampen a paper towel with 10% bleach to clean gloves between samples

5 **Labeling & Organization**· 5m

- Markers for labeling
- Label the tubes as follows:
  - **Left side:** Date and operator's initials
  - **Top:** Sample order (e.g., 1, 2, 3) + "NA" =nucleic acids + full sample name
  - **Right side:** Leave blank for DNA concentration in case you have the equipment to quantify

6 Are you running the LAMP-PCR right after? If so,... 30s

- Make sure you have enough master mix vials pre-stored at -20 °C.
- Thaw the SYBR Green at 4 °C.

Cell lysis

12m 25s

7 Add **300mL of fresh whole blood in an EDTA** to 2mL microcentrifuge tube — 10s

8 Add **500 µL of CTAB lysis** buffer to the whole blood. — 10s

9 **5 seconds Vortex** thoroughly to mix. — 5s

10 Incubate at **65°C for 10** minutes — 10m 30s

11 **1 min on ice** to improve protein precipitation — 1m 30s

Protein precipitation

3m 10s

12 Add **500 µL** of **5 M** NaCl to the lysed sample by pipetting three times. — 10s

13 **5 seconds Vortex** — 10s

14 Centrifuge at **12,000x g for** 2 minutes. — 2m 30s

15 Carefully **pipette 1 mL of the supernatant** from top to bottom, ensuring that the pellet is not touched. — 10s

16 **Transfer** the 1 mL supernatant to a **new, pre-labeled 2 mL tube.** — 10s

Nucleic acids precipitation

7m 40s

17 Add **1mL of cold isopropanol** to the recovered supernatant

10s

18 **Gently invert** to mix 20 times

1m 30s

Note: At this point, you should see debris floating in the solution.

Trick: *If you are working with multiple samples, place the tubes in the rack. Use the palms of your hands to make a sandwich, and gently rotate from right to left to mix the isopropanol*

19 ***Trick:*** *If you did not see precipitation during the previous step, incubate the sample on ice or -20 °C for 10 minutes before proceeding.*

20 Centrifuge at **12,000xg for 5 minutes**

5m 30s

21 Carefully **remove the supernatant** by pipetting, making sure not to disturb the pellet stuck to the side at the bottom of the tube. Since the DNA is concentrated in the pellet, you can safely discard any blood debris present in the upper phases.

30s

Nucleic acids clean up and DNA quantification

18m 43s

22 Add **1 mL of 70% ethanol** to wash the pellet

10s

23 **Vortex** for 1 second.

3s

24 Centrifuge at **12,000x g for 2 minute**s

2m 30s

25 **Discard** the ethanol by inverting the tube and **tapping** it repeatedly on a paper towel until any remaining ethanol drains down the side.

Note: It is recommended to invert the tube upside down only once. While inverting, gently rotate the tube to prevent the liquid from putting pressure on one side of the tube.

26 Add **1 mL of 100% ethanol** to wash the pellet — 10s

27 **Discard** the ethanol by inverting the tube and **tapping** it repeatedly on a paper towel until any remaining ethanol drains down the side. — 20s

28 Dry the DNA for **3 minutes at 70 °C.** — 3m 10s

29 **Resuspend** the dried pellet in preheated 25-50 µL by pippeting 20 times. — 30s

30 **Incubate at 70 °C for 5 minutes** to improve DNA dissolution — 5m 10s

31 **Vortex** for 5 seconds — 10s

32 **Centrifuge** at 12000 for 1 minute — 1m 10s

33 Quantify DNA by **NanoDrop,** taking DNA from the top of the solution. — 5m

Sample storage — 1m

34 **Store** at 4°C for short-term (< 1week) or -20°C for long-term storage (>1 week). — 1m

Running LAMP- PCR — 1h 22m

35 Take out the LAMP-PCR mix stored at -20°C and spin it down. — 1m
Note: include a negative and positive control.

36 Add **2 µL of SYBR Green** to the lid. 10s

37 Load 5 µL of pre-extracted DNA and pipette 5 times. 20s

38 Incubate for 1 hour at 65 °C. 1h 20m

39 Spin it down and record the result: brown = negative, green = positive. 30s