Sep 09, 2020

FloodLAMP LAMP Assay Protocol v4.1 (0.5ml, Strips, Multichannel)

Randy True[1]

[1]FloodLAMP Biotechnologies PBC

1 Works for me dx.doi.org/10.17504/protocols.io.bkvnkw5e

FloodLAMP.bio XPRIZE Rapid Covid Testing

Randy True

ABSTRACT

FloodLAMP uses an assay chemistry developed by Brian Rabe and Prof. Connie Cepko at The Cepko Lab at Harvard Medical School. "Rabe Cepko," as it's known, uses an ultra cheap, streamlined front end (sample inactivation and RNA purification+concentration). This protocol has been chosen by many groups, from pharmaceutical companies to researchers working in the developing world. The same aspects that make it ideal for Africa also make it ideal for America—it's cheap, works well, and can be implemented by any basic chemistry or biology lab.

This assay was validated clinically in May by researchers at Mass General Hospital. In addition, the key amplification reagent (NEB Colorimetric LAMP Master Mix) is the same product used in the FDA-approved test by Color Genomics, who does about 5K tests per day in the SF Bay Area.

Our current preferred version incorporates a transfer of the nucleic acid bound silica in ethanol to the PCR tubes (strips or plates) where the LAMP reaction is carried out. The pellet resuspends easily in the 80% ethanol and—once in the PCR tubes—can be quickly dried on a heat block. Then they are ready for addition of the 1X LAMP Reaction Mix. We have used alternatives to this method, including an elution of the nucleic acid and also a resuspension of the pellet in water or 1X PBS, then addition to the LAMP reaction. With the LAMP Master Mix (NEB 1804), the assay uses 2 prepared solutions: a NaI Binding Solution and the Glass Milk (also called "prepared silica").

On our website are our protocols in worksheet form as we use in the lab. This and more information will be coming soon.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

[1] Rabe B, Cepko C. SARS-CoV-2 Detection Using an Isothermal Amplification Reaction and a Rapid, Inexpensive Protocol for Sample Inactivation and Purification. medXriv preprint 4-28-20 https://www.medrxiv.org/content/10.1101/2020.04.23.20076877v1

DOI

dx.doi.org/10.17504/protocols.io.bkvnkw5e

PROTOCOL CITATION

Randy True 2020. FloodLAMP LAMP Assay Protocol v4.1 (0.5ml, Strips, Multichannel). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bkvnkw5e

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

[1] Rabe B, Cepko C. SARS-CoV-2 Detection Using an Isothermal Amplification Reaction and a Rapid, Inexpensive Protocol for Sample Inactivation and Purification. medXriv preprint 4-28-20 https://www.medrxiv.org/content/10.1101/2020.04.23.20076877v1

LICENSE

Citation: Randy True (09/09/2020). FloodLAMP LAMP Assay Protocol v4.1 (0.5ml, Strips, Multichannel). https://dx.doi.org/10.17504/protocols.io.bkvnkw5e

CREATED

Sep 04, 2020

LAST MODIFIED

Sep 09, 2020

PROTOCOL INTEGER ID

41614

GUIDELINES

Individuals are responsible for the chemical and bio safety training to safely complete this protocol.

MATERIALS

NAME	CATALOG #	VENDOR
Triton X-100	T8787-50ML	Sigma Aldrich
Hydrochloric acid	320331-500ML	Sigma – Aldrich
Sodium hydroxide	S8045	Sigma – Aldrich
UltraPure Distilled Water	10977015	Thermo Fisher Scientific
UltraPure™ 0.5M EDTA, pH 8.0	15575020	Thermo Fisher
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP)	C4706	Sigma Aldrich
Guanidine hydrochloride	G3272-1KG	Sigma Aldrich
Sigma 100% Ethanol	E7203	
Sodium Iodide (NaI)	793558	Sigma
Silicon Dioxide	SI108	Spectrum Chemicals
Phosphate Buffered Saline	10010023	Thermofisher
10X Primers (As1e and N2 combined)		IDT Technologies

MATERIALS TEXT

Tubes:
- USA Scientific TempAssure 0.2 mL, 8 pieces PCR Tube Strips w/ Optical Flat Cap Strips ($83 for 125 strips)
- 1.5mL Eppendorf DNA LoBind Tubes ($39 for 250)
- 5mL Eppendorf DNA LoBind with screw cap ($79 for 200)
- 5mL Eppendorf with screw cap, amber ($85 for 200)
- 5mL Eppendorf DNA tube with snap cap ($58 for 200)
- 30mL Self Stading Tubes - Chubs from Stellar Scientific ($99 for 500)

Reagents (see website for more information):
- Zeptometrix Inactivated virions (NATSARS(COV2)-ST)
- SARS-CoV-2 RNA Control 1, Twist RNA (102019)

SAFETY WARNINGS

Both TCEP and EDTA should be handled cautiously as they can cause severe eye damage and are toxic if inhaled. See SDS for TCEP, EDTA, NaOH, HCl and NaI for more safety information.

BEFORE STARTING

- Set Up: turn on heat block to 65C with PCR tube block inside
- Safety Procedures: always wear appropriate PPE

Purification (.5mL sample) 14m

Citation: Randy True (09/09/2020). FloodLAMP LAMP Assay Protocol v4.1 (0.5ml, Strips, Multichannel). https://dx.doi.org/10.17504/protocols.io.bkvnkw5e

1 Spike selected samples with Twist RNA 1m

2 For each 8 samples, Add 45uL of glass milk (GM) to 2.25mL of binding solution (BS), vortex glass milk before using, flicking tube to be sure that there is no pellet at the bottomand it's evenly mixed 30s

3 Add 255uL of BSGM to 500ul samples, while adding BSGM pipette up and down 3x at bottom, 3x in middle, draw from middle. Start 10min timer after last addition. 2m

4 Vortex samples 3sec, before adding to rocker or rotator. Alternatively shake or vortex every 2min. 10m

5 Spin down samples for 1min 30s

Express Wash and Transfer 24m

6 Pour the supernatant into a "sample supernatant" 50ml Falcon tube (or other waste)

7 Gently add 900uL of 80% EtOH, do not disturb the pellet (predraw about 100ul of air so next aspirate removes all liquid)

8 Aspirate all of the EtOH using the same tip and discard in "ethanol supernatant" 50ml Falcon tube (or other waste)

9 Add 100uL 80% EtOH, resuspend the pellet and transfer to labeled PCR strip

10 When all are in PCR strips, with P200 multichannel pipette, transfer 20uL from each to 2nd PCR strip. Cap all strips. 2m

11 Spin down strips for 1min 1m

12 Aspirate supernatant with 200uL multichannel pipette, hugging side of tubes away from pellet 1m

13 Heat strips on heat block set at 65C for 5-10 minutes (open covered with foil), the pellet should look chalky and not wet 10m

Citation: Randy True (09/09/2020). FloodLAMP LAMP Assay Protocol v4.1 (0.5ml, Strips, Multichannel). https://dx.doi.org/10.17504/protocols.io.bkvnkw5e

LAMP Reaction 40m

14 Make the ALL MasterMix, two separate tubes for R and AN primers 3m

14.1 For each strip of 8, add 66uL dH2O, 22uL of 10X Guanidine, vortex 3s

14.2 Add 22uL of the 10x primers (AN for 80% pellet strip and R for 20%), vortex 3s

14.3 Add 110uL of LAMP MM, vortex 3s

15 Add 25uL x n strips + 2uL (27, 52, ...) of the ALL MM to each tube in a new PCR strip, one strip for R and one for AN 1m

16 With multichannel pipette, add 25uL of ALL MM to each of the pellet strips using the multichannel 200uL pipette (AN for 80% pellet strip and R for 20%), pipet up and down 5x 5m

17 Cap strip tubes, remove from holder, check caps, flick to mix, snap down 1m

18 Incubate strip tubes on heat block at 65C for 25min (can check at 20min and 30min) 30m

19 Observe tube color, should be bright pink. Note if orange tinted (likely pellet was not dry).

20 Remove strips, let cool for at least 1min before snapping photo.

Citation: Randy True (09/09/2020). FloodLAMP LAMP Assay Protocol v4.1 (0.5ml, Strips, Multichannel). https://dx.doi.org/10.17504/protocols.io.bkvnkw5e