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FloodLAMP Inactivation Protocol v3.1

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Works for me

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FloodLAMP.bio

XPRIZE Rapid Covid Testing



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ABSTRACT

The inactivation protocol uses a chemical plus heat to break open cells (including virus, if present) and preserve the RNA. The inactivation can be done outside a lab setting, at the point of sample collection or drop-off. This is our preferred mode, and we refer to these as "inactivation stations". Prior to inactivation, the samples may contain live, contagious virus, so it is crucial that personnel use proper PPE and safe handling procedures. Many current community screening efforts are performing non-lab based sample processing (for example, [Dave O'Connor](#) and [Chris Mason](#)), and we have followed their examples with the use of splash guards in addition to PPE.

After inactivation, samples should be refrigerated or stored on ice before same-day processing through the assay.

On our [website](#) are our protocols in worksheet form as we use in the lab. This and more information will be live 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. medRxiv preprint 4-28-20
<https://www.medrxiv.org/content/10.1101/2020.04.23.20076877v1>

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PROTOCOL CITATION

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

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GUIDELINES

Individuals are responsible for the chemical and bio safety training to safely complete this protocol. Be careful of aerating samples pre-inactivation. The inactivation solution should also be handled cautiously.

MATERIALS

NAME	CATALOG #	VENDOR
Sodium hydroxide	S8045	Sigma – Aldrich
UltraPure 0.5M EDTA, pH 8.0	15575-038	Thermo Fisher Scientific
UltraPure Distilled Water	10977015	Thermo Fisher Scientific
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP)	C4706	Sigma Aldrich
Sigma 100% Ethanol	E7203	
Phosphate Buffered Saline	10010023	Thermofisher

MATERIALS TEXT

Tubes:

- 1.5mL Eppendorf DNA LoBind Tubes (\$39 for 250)
- 5mL Eppendorf LoBind with screw cap (\$79 for 200)
- 5mL colorless Eppendorf DNA tube with screw cap (\$65 for 200)
- 5mL amber Eppendorf DNA tube with screw cap (\$85 for 200)
- 5mL Eppendorf DNA tube with snap cap (\$58 for 200)
- 30mL Self Stading Tubes - Chubs by Stellar Scientific (\$99 for 500)

Reagents:

- Zeptomatrix

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 dry heater, get out frozen lab armor, turn on centrifuge, make temp QC tube for each size and volume of tube going through inactivation process
- Safety Procedures: always cover heater with plastic cover, wear appropriate PPE

Sample Preparation

2m

2m

- 1 For dry nasal swabs add 2.5mL 1xPBS, soak swabs for 2minutes, remove and discard swabs properly
- 2 For positive controls, spike selected samples with inactivated virions

Inactivation

20m

- 3 Spray all closed tubes with 70% ethanol
- 4 Vortex the 100x Inactivation Solution within five minutes of use

- 5 Add 100x Inactivation Solution to collection tube, add 50uL per 5mL collected sample 2m
- 6 For each sample, vortex for 5 seconds, shake and then vortex for another 5 seconds
- 7 Heat the samples at 95C for 8 minutes, be sure to add the QC tube(s) for in situ temperature check 8m
- 8 Chill samples using the frozen lab armor for 4 minutes, alternatively on ice 4m
- 9 Check caps are tight, then Centrifuge samples at 5K rpm for 4 minutes 4m
- 10 Aspirate the clarified samples into new tubes, be sure not to disturb any solids at the bottom of the original tube 2m