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FloodLAMP Inactivation Protocol v3.1

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Manuscript citation:

[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

Protocol status: Working
We use this protocol and it's working

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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.

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

MATERIALS

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

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:
- Zeptometrix

Troubleshooting

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 start

- Set Up: turn on dry heater, get out fozen 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

1 For dry nasal swabs add 2.5mL 1xPBS, soak swabs for 2minutes, remove and discard swabs properly — 2m

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, alteratively 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