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FloodLAMP Inactivation Solution Prep v1.0

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Protocol status: Working

We use this protocol and it's working

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Abstract

The Inactivation Solution includes TCEP, EDTA, NaOH, and water. It should be made in a chemical fume hood, as the TCEP is caustic. It is very straightforward to make if starting with liquid-form TCEP. We have not made it with powder-form TCEP, which is about 30% cheaper at low volume retail prices from Sigma.

We have yet to do long-term stability studies; however, we think it's fine stored at room temperature for at least a few weeks. If yellow Binding Solution does not go clear upon addition to an inactivated sample or PBS+Inactivation Soln, suspect that the Inactivation Solution may have degraded, and repeat with another aliquot or freshly-made Inactivation Solution.

It has also been suggested by Albert Yu of Brandeis has recommended using a 1.4N NaOH concentration for saliva instead of 1.15N NaOH. NaOH should be scale up by 1.4/1.15 and water down for this.

Guidelines

Individuals are responsible for the chemical safety training to safely complete this protocol. This procedure should be completed in a chem fume hood with appropriate PPE.



Materials

MATERIALS

⊗ UltraPure® DNase/RNase-Free Distilled Water **Thermo Fisher Catalog #10977023**

⊗ UltraPure® 0.5M EDTA, pH 8.0 **Thermo Fisher Catalog #15575020**

⊗ Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C4706**

⊗ Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #X100-100ML**

⊗ Sodium hydroxide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #306576**

Tubes:

- 5mL colorless Eppendorf DNA tube with screw cap (\$65 for 200)
- 30mL Self Stading Tubes - Chubs by Stellar Scientific (\$99 for 500)

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, and NaOH for more safety information.



Preparation

5m 30s

- 1 Get amounts of TCEP, EDTA, NaOH and Ultrapure at volumes shown in chart below:

For 1.15N Final NaOH Concentration

Final Volume	4 ml	20 ml
TCEP Volume (0.5M)	2 ml	10 ml
EDTA (0.5M)	800 ul	4 ml
NaOH (10N) > 1.15N final	460 ul	2.3 ml
Ultrapure dH2O	740 ul	3.7 ml

- 2 Add all TCEP to tube, break glass vial tops off, using the 1000uL pipette extract the liquid with the vial inverted 2m
- 3 Add EDTA 1m
- 4 Vortex for 10s, be sure the tube is fully closed 10s
- 5 Add NaOH 1m
- 6 Vortex for 10s, be sure the tube is fully closed 10s
- 7 Add UltraPure dH2O 1m
- 8 Vortex for 10s, be sure the tube is fully closed 10s