Sep 08, 2020

Freeze-drying (Lyophilization) of CoronaDetective tubes

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1 Works for me dx.doi.org/10.17504/protocols.io.bk44kyyw

XPRIZE Rapid Covid Testing Guy Aidelberg

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ABSTRACT

A protocol for freeze-drying (Lyophilization) of CoronaDetective tests, and more generally any QUASR RT-LAMP reaction.

The amounts here are for one 96 well standard PCR plate and for **20 µl** reactions

This can be scaled for any amount and size.

EXTERNAL LINK

https://app.jogl.io/project/181

DOI

dx.doi.org/10.17504/protocols.io.bk44kyyw

PROTOCOL CITATION

Guy Aidelberg, Rachel Aronoff 2020. Freeze-drying (Lyophilization) of CoronaDetective tubes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bk44kyyw

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https://app.jogl.io/project/181

KEYWORDS

LAMP, Open Science, Covid19, Sars-Cov2, RT-LAMP, Lyophilization , Freeze-drying

CREATED

Sep 08, 2020

LAST MODIFIED

Sep 08, 2020

PROTOCOL INTEGER ID

41852

GUIDELINES

The amounts here are for one standard 96 well PCR plate (12 experiments), and for **20 µl** reactions

This can be scaled for any amount of plates at any reaction volume.

MATERIALS

NAME	CATALOG #	VENDOR
Isothermal Amplification Buffer - 6.0 ml	B0537S	New England Biolabs
Magnesium Sulfate (MgSO4) Solution - 6.0 ml	B1003S	New England Biolabs
Recombinant RNasin(R) RNase Inhibitor, 10,000u	N2515	Promega
Deoxynucleotide (dNTP) Solution Mix	N0447S	New England Biolabs
2019-nCoV_N_Positive Control	10006625	Integrated DNA Technologies
Bst 2.0 Warm Start DNA Polymerase Glycerol-free	M0402B	New England Biolabs
D-( )-Trehalose dihydrate	T5251	Sigma Aldrich
WarmStart® RTx (Glycerol-Free)	M0439B-HC1	New England Biolabs

EQUIPMENT

NAME	CATALOG #	VENDOR
Scientific Freeze Dryer	HRFD-Med-Sci-EU	

SAFETY WARNINGS

There is no biohazard risk from producing these freeze-dried tubes, but care must be taken to avoid any potential contamination with target sequences or RNAse.

Best practices should be followed (appropriate PPE, RNaseAway, etc.).

BEFORE STARTING

Make sure to have all needed primers and reagents in sufficient quantities.

Fluorescence-tagged primers and complementary quencher sequences are essential for QUASR detection.

Standard Enzymes usually come in 50% Glycerol, so as to be stable in the -20C freezer. The glycerol interferes with freeze-drying.

Make sure your enzymes are Glycerol-free and stored at -80C.

Prepare the Harvest Right Lyophilizer system (or similar) for its freeze-drying run (Clean and Make sure vacuum is pulling)

Other systems are possible, and robotics are useful for scaling.

1

Thaw components (dNTPs, 10X Primer mixes (need to be made), $MgSO_4$, Isothermal amplification buffer, and Enzymes)

Vortex and quickly spin tubes down before opening for dispensing.

This protocol is for one standard 96 well PCR plate and can be scaled as needed.

1.1 **10X Primer mix:** assuming your primer stocks are at [M] **100 Milimolar (mM)** for **200 µl** add together

From: https://www.nature.com/articles/s41587-020-0513-4 (Supplementary Data 2)

For the **NM SARS CoV 2** primer set:

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Fam-FIP/BIP [M]**16 Micromolar (µM)** - **32 µl each**

LB/LF [M]**8 Micromolar (µM)** - **16 µl each**

F3/B3 [M]**2 Micromolar (µM)** - **4 µl each**

Anti-FIP-Q [M]**24 Micromolar (µM)** - **48 µl**

48 µl DNAse/RNAse free water

For the **RNAseP internal control** primer set:

FIP/BIP [M]**16 Micromolar (µM)** - **32 µl each**

LB/Hex-LF [M]**8 Micromolar (µM)** - **16 µl each**

F3/B3 [M]**2 Micromolar (µM)** - **4 µl each**

Anti-LF-Q [M]**0 Micromolar (µM)** **24 µl**

72 µl DNAse/RNAse free water

2 **FD QUASR RT-LAMP Mastermix:** In a 2ml tube mix together

280 µl dNTPs [M]**10 Milimolar (mM) each dNTP** +

200 µl of each 10x Primer Mix (NM and RNAseP From step **1.1**)+

10.66 µl Glycerol free Bst 2.0 WarmStart® DNA Polymerase [M]**120000 U/ml** +

16 µl Glycerol Free WarmStart® RTx Reverse Transcriptase [M]**75000 U/ml**

[M]**1000 U/ml** +

400 µl Trehalose [M]**50 Mass Percent** (for a 50% trehalose solution, mix 200mg trehalose with 400uL H_20) +

443 µl DNAse/RNAse free water

Vortex all or mix by pipetting up and down and then spindown.

2.1 **Rehydration Buffer:** Either now or at a later time create the rehydration buffer. For a whole plate, mix

200 µl 10X Isothermal Amplification Buffer +

100 µl Magnesium Sulfate (MgSO4) Solution [M]**100 Milimolar (mM)** +

1300 µl DNAse/RNAse free water ,

store in a cool dark place or in a fridge (**4 °C**) (stable for shipment)

3 **Dispensing:** In each well of a PCR plate place **16 µl** of the **Mastermix** from step **2**

A digital dispenser or liquid handling robot is useful here for larger scales

3.1 ***Controls:*** *Optionally, add* **4 µl** *of Internal controls (Such as IDT*2019-nCoV_N_Positive Control*) to selected tubes and mark them as such.*

4 **Sealing and piercing:** Seal the plate(s) with either foil or parafilm and then make a small puncture in the seal of each

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tube.

This is done when reactions are freeze-dried in order to prevent the small pellets from “jumping” out of the tubes under vacuum.
A 96-Pin Replicator is perfect for this but a multi-pipette, toothpick, tip, scalpel, or scissors will do.

5 **Freeze-drying:** Depending on your freeze drier, you might need to now freeze the tubes, and make sure they remain frozen (such as by placing in a frozen metal rack or touching a frozen metal block).
Otherwise, simply place in a freeze-drier and run overnight or until done.
We start by freezing to ~ **-40 °C** for a few hours
then turn on the vacuum (aiming for 500mtorr) for another couple of hours,
then slowly heating by **10 °C** every hour with the vacuum still on.

Scientific Freeze Dryer
lyophilizer
Harvest Right HRFD-Med-Sci-EU

6 **Storage:** Make sure each tube has a similarly sized dried pellet and reseal the plate with either film, foil, or caps.
Store in a dark and dry place preferably in a sealed bag with a desiccant. (stable for shipment)

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