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WarmStart LAMP®

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Kenneth Schackart¹

¹University of Arizona

Yoon Lab



Kenneth Schackart

University of Arizona

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Protocol status: Other

Refer to the actual protocol instead. I had to publish this to free up space in Yoon Lab.

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Abstract

How to run nucleic acid amplification using the Warm-Start LAMP kit.

Each reaction produces 25 μ L.

For the original protocol, look at:  LAMP Protocol.pdf .

*I have not verified the details of this protocol, I would like to remove it, but I cannot since it has been forked. If you would like to use this protocol, I would recommend using the PDF above instead.

Guidelines


Gloves must be worn at all times.

Use all precautions to avoid contamination when making reaction mixture.

Always pipette mix each reagent in aliquot before pipetting.

Materials

MATERIALS

 WarmStart LAMP Kit (DNA and RNA) - 100 rxns **New England Biolabs Catalog #E1700S**

 DNase/RNase free distilled water **Thermo Fisher Scientific Catalog #10977023**

- 70% ethanol solution in DI water
- RNAway
- WarmStart LAMP Master Mix (2x)
- LAMP primer mix (10x)
- Target DNA or RNA
- RNase free water


Troubleshooting



Prepare Work Area



- 1 Spray entire work area with 70% EtOH including pipettes, tip holder used for holding PCR tubes, and work surface. Wipe with a paper towel.
- 2 Spray entire work area with RNAway.

Gather Materials

- 3 Take styrofoam container to Marley 527 (directly across from Marley 509) and fill halfway with ice.
- 4 Set PCR tube holder on ice, and allow to cool for  00:03:00 .
- 5 Transfer Master Mix, primers, RNase free water, and target tubes from freezer to PCR tube holder on ice.
- 6 Allow reagents to thaw on ice
- 7 Carefully obtain (2) 0.2 mL PCR tubes. Label one with "NTC" and the other "TARG". These will be your reaction vessels.

Note

To avoid contamination when grabbing PCR tubes, only touch the outside of tubes. Avoid touching the inside of the caps of other tubes in this process. This is critical.


- 8 Vortex mix all reagents for approximately  00:00:05 .
- 9 Spin down all reagents for approximately  00:00:05 .

Prepare Reaction



- 10 Using a 2-20 μL pipette, transfer  9 μL RNase free water into the **TARGET** reaction vessel.
Using a 2-20 μL pipette, transfer  10 μL RNase free water into the **NTC** reaction vessel.

- 11 Using a 2-20 μL pipette, transfer  12.5 μL WarmStart Master Mix into each reaction vessel.

- 12 Using a 0.5-10 μL pipette, transfer  2.5 μL Primer to each reaction vessel.

Note

Primer will depend on what your target is. For E. Coli OH157, the primer tube is labelled with an 'F'.

- 13 Using a 0.5-10 μL pipette, transfer  1 μL Target into the **TARGET** reaction vessel.

- 14 Vortex mix the reaction mixture.

- 15 Spin down reaction mixture.

Run LAMP Reaction

- 16 Place reaction vessels into thermocycler.
- 17 Turn on thermocycler
- 18 Hit PROCEED to select a reaction cycle.
- 19 Scroll using the '<' and '>' keys to get to LAMP3.



20 Press PROCEED to begin