

Feb 20, 2019 Version 1

# PCR Reaction Optimization V.1



Forked from WarmStart LAMP®

DOI

[dx.doi.org/10.17504/protocols.io.yb9fsr6](https://dx.doi.org/10.17504/protocols.io.yb9fsr6)

Kenneth Schackart<sup>1</sup>

<sup>1</sup>University of Arizona

Yoon Lab



Kenneth Schackart

University of Arizona

---

OPEN  ACCESS



DOI: [dx.doi.org/10.17504/protocols.io.yb9fsr6](https://dx.doi.org/10.17504/protocols.io.yb9fsr6)

**Protocol Citation:** Kenneth Schackart 2019. PCR Reaction Optimization. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.yb9fsr6>

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** In development

**We are still developing and optimizing this protocol**

**Created:** February 19, 2019

**Last Modified:** February 20, 2019

**Protocol Integer ID:** 20577



## Abstract

How to run nucleic acid amplification using the Thermo scientific PCR Master Mix kit.  
Each reaction produces 50  $\mu$ L.

For the original protocol, look at:  [PCR Master Mix Manual.pdf](#).

## 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

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


- 70% ethanol solution in DI water
- RNAway
- Thermo Scientific Master Mix (2x)
- PCR primer mix (25  $\mu$ M)
- Target DNA or RNA
- RNase free water



## 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

## Gather Materials

- 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 Add the following to your two tubes:

	Target	NTC
PCR Master Mix	25 $\mu$ L	25 $\mu$ L
Primer Mixture (25 $\mu$ M)	0.2 - 2.0 $\mu$ L	0.2 - 2.0 $\mu$ L
Target	1 $\mu$ L	-
Water	to 50 $\mu$ L	to 50 $\mu$ L
Total	50 $\mu$ L	50 $\mu$ L

▮ Various primer concentrations are to be optimized. Start with 1.0  $\mu$ L (0.5  $\mu$ M).

- 11 Vortex mix the reaction mixture.

- 12 Spin down reaction mixture.

## Run LAMP Reaction

- 13 Place reaction vessels into thermocycler.

- 14 Turn on thermocycler

- 15 Hit PROCEED to select a reaction cycle.

- 16 Scroll using the '<' and '>' keys to get to PCR.

- 17 Press PROCEED to begin