Jun 13, 2025

Primer Preparation

DOI
https://dx.doi.org/10.17504/protocols.io.n92ldy1e7l5b/v1

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Protocol Citation: Dakota Betz 2025. Primer Preparation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldy1e7l5b/v1

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

Created: June 05, 2020

Last Modified: June 13, 2025

Protocol Integer ID: 37883

Keywords: primer preparation dilute primer stocks for use, primer preparation dilute primer stock, pcr reaction, primer, pcr, preparation

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Our protocols are constantly evolving and old versions will be deleted.
The documents here are not intended to be cited in publications

Abstract

Dilute primer stocks for use in your PCR reactions.

Before start

Use 75% Ethanol to clean the lab bench, tube racks, and pipettes before taking out any reagents and starting the protocol.

Clean lab area first!

1 Check that you have cleaned the lab bench, tube racks, and pipettes with **75% Ethanol** before taking out any reagents and starting this protocol.

Note

Be especially aware of potential contamination sources whenever you are dealing with reagents that are part of a stock. Contaminating the stock ruins it for the entire lab!

Primer Preparation

2 Obtain a *new* **water aliquot** from the -20 frost-free freezer and place in your cleaned tube rack to thaw.

3 Primer stocks are located in the -80 freezer (right side of shelf 2). Obtain *only* the primer stocks you need right now from the box, then place the box immediately back in the freezer to avoid thawing more primer stocks than necessary. Place the primer stocks you'll be using (forward and reverse) in your clean tube rack to thaw.

4 While the stocks/water thaw, label one clean/new microcentrifuge tube for the forward primer and one for the reverse primer. Include the 1:10 concentration info, name of the primer, and today's date.

5 Use the following table to prepare the amount of stock you need. Aliquot water into your labeled microcentrifuge tubes first. Once the primers are thawed, be sure to mix them thoroughly (vortex) before pipetting the appropriate amount into your microcentrifuge tubes. *Also be careful not to mix forward and reverse primers: use only one stock per microcentrifuge tube, labeled accordingly.*

	Approx. # PCR Reactions	Water (uL)	Primer Stock (uL)	Total Volume (uL)
	Up to 50	45	5	50
	Up to 100	90	10	100
	Up to 200	180	20	200

6 Mix thoroughly (vortex) newly prepped primers. You can store these in your labeled PCR box in the -20 frost-free freezer. Be sure to return primer stocks to their appropriate box in the -80 as soon as you are finished.