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We use this protocol and it's working

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Primer stock preparation

Brian Lovett[1], Kristen Pierce[2]

[1]USDA-ARS; [2]West Virginia University

Lovett Lab - USDA ARS EPPRU

Brian Lovett
USDA-ARS

ABSTRACT

General protocol for preparation of lyophilized primer stocks from IDT.

GUIDELINES

Recommended: When you receive your lyophilized primers, check the name of the primer, primer sequence and the concentration listed on the tube. IDT provides a specification sheet you can download/archive for more information.

MATERIALS

Lyophilized IDT primers, molecular grade water, pipettes and pipette tips.

Keywords: lyophilized primer reconstitution, primer dilution

Preparation of stock solution

1 Reconstitute lyophilized primers in required volume of molecular grade water (i.e., RNase/DNase free) to [M] 100 micromolar (µM).

1.1 Practically, the microliters of water required to create a 100 µM solution is 10x the nmol concentration of lyophilized primer (i.e., if [M] 23.5 Nanomoles is noted on side of tube, then add 235 µL of water to stock tube).

2 After water has been added, vortex for at least 00:00:10 to ensure the lyophilized primers are fully dissolved. 10s

3 Once reconstituted, the stock solution can be stored in a labeled box at -20 °C, and thawed completely before use.

Preparation of working solution

4 Allow stock solution to thaw completely at Room temperature.

4.1 While waiting for your stock to thaw, prepare clean microcentrifuge tubes by labeling them with the primer name and date of preparation. Label the lid so it is easy to identify tubes in freezer box.

5 Vortex the stock solution for at least 00:00:10 to ensure homogenization before adding it to the working solution. 10s

6 To prepare a [M] 10 Molarity (m) working solution from the [M] 100 micromolar (µM), you will dilute 1:10 (i.e., 1 µL of stock for every 9 µL of water).

6.1 For a 100 µL working solution, pipette 90 µL of molecular grade water into the newly labeled microcentrifuge tube and then add 10 µL of the stock solution.

7 Once the concentrated stock is added, vortex the new working solution for at least 00:00:10 10s and store at -20 °C to use as needed.

Use of primers

8 For a typical 25 µL PCR reaction, 1 µL of each primer is used (i.e., [M] 0.4 micromolar (µM) final concentration).

8.1 To maintain optimal use of your primers, limit freeze/thaw cycles, as this can lead to degradation over time.