Primer resuspension (DNA olionucleotides in TE buffer)

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Abstract

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Protocol

Gather supplies and Label

Step 1.
Gather supplies
- IDTE pH 7.5 (1X TE Solution) in 50 mL conical tube
- pipette and tips
- DNA oligonucleotides (mass and molecular weight)
- 1x PCR or microcentrifuge tube

Label tubes
- Apply IDT label to primer tube and write '100 uM in TE'
- To microcentrifuge tube apply label and write '10 uM in TE'

Calculate volume of TE for 100 uM suspension of primers

Step 2.

Our goal is to make a stock solution of primers at 100 uM

Calculations:

n = amount of oligo (nmoles)

C = desired concentration (uM = 1 umol/L = 1 nmole/mL)

V = volume of TE to add (uL)

V=n/C

For example, to make 100 uM solution from 69.6 nmoles of oligo, add V=n/C=69.6 nmole / 100 nmole/mL = 0.696 mL = 696 uL

More information:

This calculation will often be given on the IDT sheet.

Resuspension and storage guidelines

https://www.idtdna.com/pages/decoded/decoded-articles/core-concepts/decoded/2011/03/16/dna-oligonucleotide-resuspension-and-storage

Resupension calculator

https://www.idtdna.com/calc/resuspension/

Calculate volume of TE to dilute 100 uM to 10 uM solution of primers

Step 3.

Our goal is to make a working solution of primers at 10 uM

Calculations:

V1 = volume of 100 uM primers (uL)

V2 = final volume of mixture (uL)

C1 = initial concentration = 100 uM

C2 = final concentration = 10 uM

V_TE = amount of 1xTE to add (uL)

C1*V1 = C2*V2

For make 100 uL of 10 uM solution, need V1=C2*V2/C1 = (10 uM*100 uL)/(100 uM) = 10 uL of solution C1

We need to calculate the amount of TE to dilute the V1 solution with

V2 = V1+V_TE

V_TE=V2-V1 = 100 - 10 uL = 90 uL

Therefore, we'll combine 10 uL of 100 uM primers with 90 uL TE to create our 10 uM primer mix

Resuspend primers to 100 uM

Step 4.

1. Spin down oligonucleotide tube in benchtop microcentrifuge prior to opening the tube for resuspension.
2. Pipette in the volume of 1xTE required for a 100 uM solution.
3. Allow to sit for 2 min, then vortex for 15 sec.
4. Final storage of primers (after dilution) in -20C freezer in stock primer freezer box

Dilute primers to 10 uM

Step 5.

1. Pipette the volume of TE buffer required for dilution (V_TE; smaller volume) into labeled microcentrifuge tube
2. Pipette the voume of 100 uM primer solution (V1) into the same tube
3. Vortex for 15 s to mix
4. Final storage of primers in -20C freezer in working primer