Primer re-nature and dilution for storage and use

Adapted from www.minipcr.com/store-and-use-pcr-primers/

Basic principle

Primer renaturation and dilution are performed after arrival in the laboratory. Primers arrive after a synthesis and lyophilization procedure and must be rehydrated before use in a polymerase chain reaction.

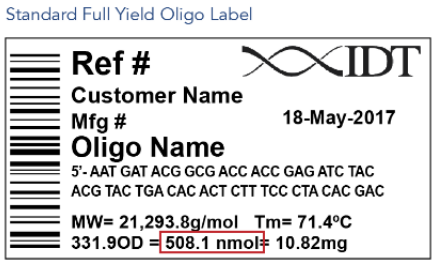
Required equipment and consumables

1. 2mL Eppendorf tubes
2. Lyophilized primer set tubes
3. Nuclease-free water
4. Pipettes
5. TE 1X buffer (10 mM Tris pH 8.0, 1 mM EDTA)
6. Tips and pipettes

Procedure

1. Make a stock solution for long-term storage

Use 1X TE buffer for the stock solution. Primers will dissolve in this buffer. The desired concentration for your primer stock is 100 µM, which is 100 to 1,000 times the concentration used in the actual PCR reaction. To determine how much 1X TE buffer needs to be added to each primer tube, check the amount of primer contained (see picture 1). It is advisable to let the pellet dissolve by storing the primer at 4°C overnight.



Picture 1. Oligo tube label. This information is also available on your IDT specification sheet, along with oligo properties and yield information.

The oligo tube label indicates the amount of lyophilized primer in the tube, reported in nanomoles (nmol). The required volume of 1X TE buffer is determined by multiplying the primer amount (e.g., 508.1 nmol) by 10. Thus, 5,081 µL of 1X TE buffer must be added to prepare a stock primer solution at 100 µM. This solution is stable and might be stored frozen for years.

2. Dilute your stock solution for the PCR working solution

The stock solution is diluted with nuclease-free water to prepare a working primer solution, which is used for every PCR run. This procedure prevents contamination and repeated freeze-thaw damage of the stock primer solution.

The working solution is prepared by diluting the primer stock 1:10 with nuclease-free water or Low EDTA TE Buffer to reduce EDTA interference during PCR. The desired concentration for your working solution is 10 µM. In a fresh and labeled 2 mL Eppendorf tube, add 90 µL of nuclease-free water and 10 µL of the rehydrated primer stock solution.

Precautions

Avoid repeated freeze-thaw cycles of the stock primer solution. Label the primer working solution, including concentration and date. Keep the same primer name as the original stock solutions.

Applications

Stock and working primer solutions are used in PCR, with applications ranging from gel verification for PCR products to sequencing.

Bibliography

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