invitrogen

Taq DNA Polymerase PCR Buffer

Cat. No. 18067-017 **Size: 2 x 1 ml**

Store at –20°C.

Description

For use with *Taq* DNA Polymerase (Cat. No. 18038). The PCR Buffer is supplied as a 10X concentrate and should be diluted 1:10 in the final reaction (*e.g.,* use 5 µl in a 50-µl PCR reaction).

Buffer Composition (10X): 200 mM Tris-HCl (pH 8.4), 500 mM KCl.

Components

10X PCR Buffer minus Mg
50 mM Magnesium Chloride

Quality Control

No detectable contaminating activity is observed in the endodeoxyribonuclease and ribonuclease assays.

Guidelines and Recommendations

Critical parameters and troubleshooting information for PCR are well documented (1).

PCR reactions should be assembled in a DNA-free environment. Use of "clean" dedicated automatic pipettes and aerosol-resistant barrier tips are recommended.

Always keep the control DNA and other templates to be amplified isolated from the other components.

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Basic PCR Protocol

The following protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of *Taq* DNA Polymerase, primers, $MgCl_2$, and template DNA) may vary and need to be evaluated by the customer.

1. Add the following components to a DNase/RNase-free 0.5-ml microcentrifuge tube sitting on ice. Scale the reaction volumes as needed. Prepare a master mix for multiple reactions, to minimize reagent loss and to enable accurate pipetting.

Components	Volume	Final Concentration
10X PCR buffer minus Mg	10 µl	1X
10 mM dNTP mixture	2 µl	0.2 mM each
50 mM $MgCl_2$	3 µl	1.5 mM
Primer mix (10 µM each)	5 µl	0.5 µM each
Template DNA	1-20 µl	-----
Taq DNA Polymerase (5 U/µl)	0.5 µl	2.5 units
Autoclaved distilled water to	100 µl	

2. Mix contents of tube and overlay with 50 µl of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.
4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. Perform 25-35 cycles of PCR amplification as follows:

   Denature 94°C for 45 seconds
   Anneal 55°C for 30 seconds
   Extend 72°C for 1 minute, 30 seconds

6. Incubate for an additional 10 minutes at 72°C and maintain the reaction at 4°C. The samples can be stored at -20° until use.
7. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining.

"Hot Start" PCR Protocol

In the "hot-start" method, the addition of *Taq* DNA Polymerase is withheld until the reaction temperature is at 80°C, to ensure high specificity of the products being synthesized.

1. Add all components as in the Basic PCR Protocol, except for the *Taq* DNA Polymerase.
2. Mix contents of tube and overlay with 50 µl of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.
4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. After denaturation at 94°C, maintain the reaction at 80°C.
6. Add 0.5 µl of *Taq* DNA Polymerase (2.5 U) to each reaction. Be certain to add the enzyme beneath the layer of oil.
7. Continue with 25-35 cycles of denaturation, annealing and extension as in the Basic PCR Protocol.

Reference

1. Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J., eds. (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, CA.

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