GenScript *Taq* DNA Polymerase Manual

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I. INTRODUCTION

Taq DNA Polymerase is a thermostable DNA Polymerase isolated from an *E. coli* strain that carries the *Taq* DNA Polymerase gene. *Taq* DNA Polymerase is the most common polymerase used for PCR* reactions. In some cases of PCR, such as RAPD PCR, adding large volume of general *Taq* DNA Polymerase (5 U/μl) will increase the glycerol concentration in the reaction mix, since the high concentration (50%) of glycerol is used in the storage buffer. High concentration of the glycerol may interfere with PCR performance. Therefore, in order to prevent from poor PCR efficiency caused by high level of glycerol, concentrated *Taq* DNA Polymerase (25 U/μl) is recommened.

II. APPLICATIONS

Taq DNA Polymerase can be used in most applications including the following:

- PCR.
- 3’ A-tailing of blunt ends.
- Primer extension.
- DNA sequencing.

III. KEY FEATURES

Key features of *Taq* DNA Polymerase:

- Terminal transferase activity. *Taq* DNA Polymerase has terminal transferase activity which results in the addition of a single nucleotide (adenosine) at 3’ end of the extension product.
- High-purity. No contamination activity has been detected in standard test reactions.

IV. SHIPPING AND STORAG

This product is shipped on blue ice. Store the product at -20°C.

V. GENERAL PCR PROTOCOL USING TAQ DNA POLYMERASE

1. Thaw all the reagents for PCR on ice. **Vortex to mix to remove concentration gradient** and then spin down briefly.
2. Since the enzyme is highly concentrated, we recommend preparing a master mix which contains appropriate units of *Taq* DNA Polymerase that can cover every individual reactions. Otherwise one can prepare storage buffer (20 mM Tris HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol) in order to dilute the enzyme into appropriate concentration.
3. Set up 50 µl PCR reaction in a thin-wall PCR tube on ice by the following recipe:
   5 µl 10X *Taq* buffer solution containing Mg^{2+}.
   1 µl 10 mM dNTP stock
   1 µl Forward primer (50 uM)
   1 µl Reverse primer (50 uM)
   2 µl Template (up to 100 ng/µl) sterile or filtered H_2O
   39.9 µl sterile or filtered H_2O
   0.1 µl *Taq* DNA Polymerase (25 units/µl)
4. Program PCR cycler as following and start:
   Initial denaturing: 94 ℃ for 3 minutes

   Then 30 cycles of: 94 ℃ for 30 seconds
   55 ℃ for 45 seconds
   72 ℃ for 60 seconds (about 1 kb/minute)

   Extension: 72 ℃ for 7 minutes
5. When the temperature of PCR cycler reaches 94°C, put PCR reaction tube in and continue the program.
6. Analyze PCR fragments on a agarose or polyacrylamide gel.

Note:

1. This is a basic protocol. One needs to optimize the reagent concentrations, conditions and parameters.
2. This protocol is for PCR cycler with a hot lid. Otherwise, mineral oil needs to be added to prevent evaporation.
3. 5% DMSO, 1M betaine, or both can be included in PCR reaction to improve the results when a GC-rich template is used.

VI. ORDER INFORMATION

Taq DNA Polymerase, concentrated, Cat. No. E00012

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