Hi-Thru *Taq* DNA Polymerase

Technical Manual No. TM0226 **Version 01222007**

I. DESCRIPTION

The kit contains reagents required for PCR* in one box combining simple handling with high flexibility. The premium quality polymerase, ultrapure dNTPs and the optimized complete reaction buffer ensure superior amplification results. The detergent-free buffer is designed for applications like DHPLC where PCR products are recommended to be free of detergents. They can also be used in microarray applications, as detergents can cause foaming when PCR products are spotted on microarray slides. The kit is recommended for use in routine PCR reactions. It is optimized for high specificity and guarantees minimal by-product formation. The buffer system is particularly suitable for plate based PCR and automated pipetting where a detergent-free buffer system is required.

II. KEY FEATURES

- Complete detergent-free, including the *Taq* DNA Polymerase and PCR buffer.
- PCR products can be directly loaded for HPLC purification without any further treatment.
- Specialized detergent free buffer system for the high throughput PCR and HPLC system.

III. CONTENTS

- Hi-Thru *Taq* DNA Polymerase (red cap): 5 units/µl Hi-Thru *Taq* DNA Polymerase in 20 mM Tris-HCl, 100 mM KCl, 0.1 EDTA, 1 mM DTT, 50% (v/v) Glycerol, pH 8.0 (25°C)
- 10× *Taq* Buffer without detergent (green cap): 100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, pH 8.3 (25°C)
- dNTP Mixture, 10 mM each, 200 µl

IV. ACTIVITY

The enzyme replicates DNA at 72°C. It catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium. It also has the extendase activity allowing TA cloning.

V. UNIT DEFINITION

One unit of enzyme incorporates 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74 °C.

VI. STORAGE BUFFER AND CONCENTRATION

Supplied in 5 units/µl in 20 mM Tris-HCl, 100 mM KCl, 0.1 EDTA, 1 mM DTT, 50% (v/v) Glycerol, pH 8.0 (25°C)

VII. STORAGE

Quality guaranteed for 12 months. Store at -20°C, avoid frequent thawing and freezing

VIII. GENERAL PCR PROTOCOL USING HI-THRU TAQ POLYMERASE

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This is a general PCR amplification protocol, optimization may be needed to get satisfactory results.

1. Set up 50 µl PCR reaction by adding the following reagents to a thin-walled PCR microcentrifuge tube or plate and mixing gently.

Reagent	Volume	Final Concentration
Water, PCR grade	40 µl	
10× *Taq* Buffer	5 µl	1×
10 mM dNTP	1 µl	200 nM each
20 µM Forward primer	1 µl	400 nM
20 µM Reverse primer	1 µl	400 nM
Template DNA	1 µl	1-100 ng per reaction
5 U/µl Hi-Thru *Taq* DNA Polymerase	1 µl	0.1U/µl
Total	50 l	

2. Suggested PCR profile:
   - 3 Step PCR
     - Initial Denaturation: 94-96°C for 2 min.
     - 25-30 Cycles of Denaturation: 94-96°C for 30 sec.
     - Annealing: 50-60°C for 30 sec. (primer T_m - 5°C)
     - Extension: 72°C for X min. (1-2.5 kb/min)
     - Final Extension: 72°C for 10 min.
   - 2 Step PCR
     - Initial Denaturation: 94-96°C for 1 min.
     - 20-30 Cycles of Denaturation: 94-96°C for 20 sec.
     - Annealing & Extension: 68°C for X min. (1-2.5 kb/min)
     - Final Extension: 72°C for 7-15 min.

IX. TROUBLESHOOTING

The table below is guideline for troubleshooting.

Problem	Probable Cause	Solution
No PCR DNA	Extension time is too short;	Hi-Thru *Taq* DNA Polymerase requires a minimum extension time of 1 minute for 1~2.5 kb template.
	One or more PCR components may be missing;	Run a positive control to make sure the PCR reaction is properly set-up.
	PCR conditions are not optimized; the annealing temperature may be too high; more cycles may be needed; the denaturation time may be too short;	Optimize the PCR conditions by decreasing annealing temperature in 2-4°C increments, or increasing the number of cycles, or increasing the denaturation time in 10 second increments. It is recommended to change only one parameter each time.
	The primers may not be designed optimally.	The primer designing is critical for high quality PCR. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Target template is highly GC-rich.	The target will be difficult to denature even with a longer denaturation step.
Non-specific DNA products	The primers may not be designed optimally.	See above.
	Annealing temperature is too low.	Optimize the PCR conditions by increasing annealing temperature in 2-4°C increments, or decreasing the number of cycles.
False positive	Reagents are contaminated.	It is recommended that a negative control without using genomic DNA be run to make sure no contamination occurs.

X. ORDER INFORMATION

Hi-Thru *Taq* DNA Polymerase, Cat. No. E00015

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