Hot Taq DNA Polymerase Kit

1. Catalog No.

Cat. No	Product	Size
K0567100	Hot Taq DNA Polymerase Kit	250U

2. Storage

1 year at -20°C

3. Contents

Component	Cat. No	Packing	Comments
Hot Taq DNA Polymerase	K0567100	250U	5 Unit/μL
10X Hot Taq PCR Buffer	K0567111	1.5ml	20mM $MgCl_2$
dNTP Mixture (2.5mM each)	K1756011	0.5ml	2.5mM each
10X Loading Buffer	K0561051	0.5ml	-
4X Magic Buffer **(Only use for High G+C content)**	K0561031	0.5ml	For High GC content

4. Description

Hot Taq DNA Polymerase, a chemically modified form of Taq DNA Polymerase, is supplied in an inactive state that has no polymerase activity at ambient temperatures. This prevents extension of nonspecifically annealed primers and primer–dimers formed at low temperatures during PCR setup and the initial PCR cycle. This makes Hot Taq DNA Polymerase ideal for quantitative real-time PCR. Hot Taq DNA Polymerase is activated by 15-minute incubation at 95°C which can be incorporated into any existing thermal-cycler program. Every lot of Hot Taq DNA Polymerase is subjected to a comprehensive range of quality control tests, including a stringent PCR specificity and reproducibility assay in which low-copy targets are amplified.

- High specificity
- Reduced non-specific amplification
- High reproducibility
- Extension rate: 2-4 kb/min at 72°C
- Half-life: 10 min at 97°C, 60 min at 94°C
- Amplification efficiency: $\geq 10^5$ fold
- 5'→3' exonuclease activity
- Extra A addition
- Contaminating nucleases: No
- Contaminating RNases: No
- Contaminating proteases: No
- Self-priming activity: No

5. Application

- Highly Specific PCR
- Low Copy Number Target PCR.(e.g. Viral Detection in Blood)
- RT-PCR of rare transcript
- Real-time PCR
- Differential Display
- Multiplex PCR, PCR-based DNA fingerprinting (VNTR, STR, and RAPD) etc.
- Degenerate PCR

6. PCR Amplification

1. Thaw 10X Hot Taq PCR Buffer, dNTP mix, primer solutions. If required, prepare 25 mM $MgCl_2$ separately. Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.

2. Add the following reagents to a thin-walled PCR microcentrifuge tube or plate.
   The master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should be included in every experiment. The optimal Mg2+ concentration should be determined empirically but in most cases a concentration of 2.0 mM, as provided in the 1X Hot Taq PCR Buffer, will produce satisfactory results. Keep the master mix on ice.

Component	Final Concentration	Volume/reaction	
10X Hot Taq PCR Buffer	1X	2 μL	5 μL
25mM $MgCl_2$ (if required)	2.0-4.0mM	Variable	Variable
dNTP mix (2.5 mM each)	200uM of each dNTP	1.6 μL	4 μL
5' Primer	0.1 - 0.5 uM	Variable	Variable
3' Primer	0.1 - 0.5 uM	Variable	Variable
Hot Taq DNA Polymerase	2.5 units/reaction	0.2 μL	0.5 μL
Distilled water	-	Variable	Variable
Template	-	Variable	Variable
Total reaction volume		**20 μL**	**50 μL**

3. Mix gently.

4. When using a thermal cycler without a heated lid, add approximately 100ul of mineral oil on top of the mixture.

5. Perform thermal cycling.

Step		Temp.	Time	Cycles
Initial Denaturation		95℃	15 min	1
Cycling	Denaturation	94℃	0.5-1 min	25-35
	Annealing	50-68℃	0.5-1 min	
	Extension	72℃	1 min (~1kb/imin)	
Final Extension		72℃	10 min	1

Note:

a. 10X Hot Taq PCR Buffer is optimized for DNA amplification condition with Hot Taq DNA polymerase. If you use Hot Taq DNA polymerase with reaction buffers supplied by other vendors, we don't guarantee good PCR results.
b. Primers should be 15 to 30 bases in length and near 50% G+C content.
c. **Magic Buffer is not necessary for normal G+C content. It will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure.** We recommend that the volume added should not exceed 25 % (v/v) of final PCR volume.