HotStart Taq DNA Polymerase

User Mannual

Cat.Nos.ZP00301(1000U)

ZP00302(5000U)

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Storage condition: The undiluted enzyme solution is stable when stored at -20℃.

Activity: 5U/ul

Storage and dilution buffer: 20mM Tris-HCl; 1mM dithiothreitol; 0.1mM EDTA; 0.1M KCl; Nonidet P40, 0.5%(v/v); Tween 20, 0.5%(v/v); glycerol, 50%(v/v); pH 8.0 (4℃).

Unit definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10mmols of dNTP into an acid-insoluble material in 30 minutes at 74℃. The reaction conditions are: 50mM Tris-HCl, (pH 9.0 at 25℃), 50mM NaCl, 10Mm $MgCl_2$, 200uM dATP, dCTP, dGTP and radiolabelled dTTP, and 12.5ug activated calf thymus DNA in a 50ul reaction.

10×Reaction buffer(with MgCl2) : 100mM Tris-HCl; 500mM KCl; 15mM MgCl2,pH9.0 (20℃). Buffer is optimized for use with 0.2mM for each of dNTPs.

Hotstart Program:95℃ 2-4min

Note: It is recommended to add dNTPs to this incubation mixture shortly before use. This is to prevent decomposition of the deoxynucleoside thiphosphate that occurs during Prolonged storage at the alkaline pH values required for optimal enzyme activity.

For example:

Reaction setup:

Component	Volume(μl)	Final Con.	Comments
10×PCR buffer	5	1×	
dNTP(10mM)	1	0.2mM	
up primer(25pmol/ul)	0.8	30~900nM	
Down primer(25pmol/ul)	0.8	30~900nM	
Sybr green I(20*)or probe	0.6ul		0.15-0.3uM
Mg2+(25mM)	3-5ul	3mM-4mM	
HotStart Taq	0.3-0.5ul	1.5-2.5U	
cDNA(ul)	Xul	10~100ng	
ddH2O(ul)	Yul	-	
Total(ul)	50	-	

Cycling program

1、Protocol for electrophoresis PCR

95℃ 4min→94℃ 30sec→（55-60）℃30sec 72℃ 1min

35-40cyclers

2、Protocol using LightCycler with Taqman probe

95℃ 4min→94℃5 sec→60℃30 sec

40cyclers

3、Protocol using LightCycler with Molecular Beacon probe

95℃ 4min→94℃5 sec→60℃20 sec→72℃20 sec

40cyclers

4、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Taqman probe

95℃ 4min→94℃ 30sec→60℃ 60sec

40cyclers

5、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Molecular Beacon probe

95℃ 4min→94℃ 30sec→60℃ 30sec→72℃30sec

40cyclers

Quality control

Each lot of hotstart Taq DNA Polymerase is tested for activity in PCR and efficient incorporation of digoxigenin-11-dUTP, and in DNA sequencing of M13mp18ssDNA. A minimum of 250 bases must be clearly legible in the sequencing gel. Each lot of Taq DNA ploymerase is tested for contaminating activities as stated below.

Test buffer: 10mM Tris-HCl; 1.5mM $MgCl_2$; 50mM KCl; pH 8.3 (20℃). 0.1mg/ml gelatin.

Absence of endonucleases: 1ul lambda DNA is incubated with hotstart Taq Dna polymerase in 50ul test buffer with a paraffin oil overlay at 65℃ for 16 hours. The amount of enzyme showing no degradation of the lambda DNA is ststed under “Endol”.

Shanghai ShineGene Molecular Bio-tech Co.,Ltd.

Add：Floor 2,Building A,328#, Wuhe Road,Shanghai,201109,China

Tel：+86-21-54460832

E-mail:master@shinegene.org.cn

Website：http://www.synthesisgene.com