Ver. HB230113

# Hieff™ Taq DNA Polymerase

## **Product description**

Hieff<sup>TM</sup> Taq DNA polymerase is a thermostable recombinant DNA polymerase expressed by *Thermus aquaticus* with a molecular weight of 94 KDa. It has  $5'\rightarrow 3'$  polymerase activity and  $5'\rightarrow 3'$  exonuclease activity but without  $3'\rightarrow 5'$  exonuclease activity. The amplified product has 3'- dA and can be directly used for TA cloning.

### Components

Components No.	Name	10101ES80	10101ES92
		(1,000 U)	(10,000 U)
10101-A	10×Taq Buffer (Mg²+ Free)	4×1 mL	4×10 mL
10101-B	25 mM MgCl <sub>2</sub>	2×1 mL	2×10 mL
10101-C	Hieff™ Taq DNA Polymerase (5 U/μL)	200 μL	2×1 mL

## **Specifications**

Polymerase	Taq DNA polymerase	
Molecular Weight	94 KDa	
	Using the activated DNA of salmon sperm as template/primer, the activity is defined as one	
Unit Definition	active unit (U) when 10 nmol of total nucleotide was ingested as acid insoluble substance	
	at 74°C for 30 min.	

## Storage

The product should be stored at  $-25^{\circ}$ C  $\sim -15^{\circ}$ C for two years.

#### Instructions

#### 1. Reaction composition (Preparation on ice)

Components	Volume(μL)	Final Concentration
ddH <sub>2</sub> O	to 50	-
10×Taq Buffer (Mg²+ Free)	5	1×
25 mM MgCl <sub>2</sub>	3	1.5 mM
dNTP Mix (10 mM each)	1 μL	0.2 mM
DNA template	Χ μL	-
Forward primer (10 μM)	2 μL	0.4 μΜ
Reverse primer (10 μM)	2 μL	0.4 μΜ
Hieff™ Taq DNA Polymerase (5 U/μL)	0.4 μL	0.04 U/μL

#### Notes:

- 1) Final concentration of  $Mg^{2+}$ : The optimal concentration of  $Mg^{2+}$  is 1.5 2 mM. If necessary, the optimal concentration of  $Mg^{2+}$  can be explored upward at intervals of 0.2 0.5 mM.
- 2) Polymerase addition: The polymerase has a certain degree of 5'- 3' polymerase activity at room temperature. In order to prevent non-specific amplification, it is suggested to add the polymerase to the reaction system in the last step.
- 3) Concentration of polymerase:  $0.04 \text{ U/}\mu\text{L}$  is recommended. It can be optimized between 0.025- $0.04 \text{ U/}\mu\text{L}$ .
- 4) Recommended use of different templates (50 μL reaction system):

Type of template	Template usage
Genomic DNA	50 ng-100 ng
Plasmid DNA	10 pg-20 ng
cDNA	1-5 μL (No more than 1/10 of the reaction system)

#### 2. Thermal cycling protocol

Stage	Temperature (°C)	Time	Cycles
Pre-denaturation	94	30 sec-5 min	1
Denaturation	94	30 sec	
Annealing	50-60	30 sec	35
Extension	72	60 sec/kb	
Final Extension	72	10 min	1

#### Notes:

- 1) Pre-denaturation temperature and time: 94°C is recommended. The recommended pre denaturation time: 30 sec for plasmid DNA and other simple templates; 3 min for complex templates such as cDNA and genomic DNA; 5-10 min for the template with high GC.
- 2) Annealing temperature and time: 60°C is recommended. Temperature gradient can be set up to find the optimum temperature for primer annealing. The recommended annealing time is set to 20 sec and can be adjusted within 10-30 sec. Too long annealing time may cause the amplified products diffusion on the agarose gel.
- 3) Amplification products: Please store the PCR amplification products at 20°C to prevent DNA degradation.

#### **Notes**

- 1. For your safety and health, please wear lab coats and disposable gloves for operation.
- 2. For research use only!