Reversible 1-min Hot Start DNA Polymerase

Cat. #: P950 (100 units), P950L (1,000 units)

Storage: Store at -20°C for up to 2 years and avoid freeze-thaw cycles. Stored at 4°C for up to 6 months.
95°C 1 minute hot start.

Product Components

Components	P950 (100 units)	P950L (1,000 units)
Reversible 1-min Hot Start DNA Polymerase (2.5 Unit / µL)	40 µL	400 µL
PCR Buffer (10 X)	500 µL	4 x 1.5 mL
$MgCl_2$ (100 mM)	100 uL	1 ml
Nuclease-Free Water	1 mL	10 mL

The composition of the 10X PCR buffer is: 100mM Tris-HCl, 500mM KCl, 15mM $MgCl_2$, PH 8.3 (at 25°C).

Application

- Fast PCR; Routine PCR; quantitative PCR.

Product Description (This product is for research use only.)

Reversible 1-min Hot Start DNA Polymerase is an optimized mixture of special Taq DNA polymerase and a proprietary inhibitor. The inhibitor **reversibly binds** to Taq polymerase at temperature below 50°C, and **completely inhibits** polymerase activity **before and after PCR reaction**, thus allowing reaction setup at ambient temperature.

1. **Fast Hot Start (30 seconds to 1 minute):** Initial thermal activation can be achieved by incubating the assembled reaction at 95°C for as short as 30 seconds. **Save up to 15 minutes** comparing to other Hot Start Taq polymerase, and therefore significantly shortens the reaction time.
2. **Exceptional sensitivity:** The polymerase can amplify low-abundance template (single-digit copies) in the reaction.
3. **Superior stability:** The polymerase activity remains the same even after being stored at 25°C for 7 days.
4. **Reduced nonspecific amplification:** Unlike traditional antibody-based Hot Start Taq polymerase, which loses its "hot start" function once activated, the inhibitor in this product will continue to inhibit the polymerase activity whenever the temperature drops below 50°C, even AFTER the PCR reaction. This unique feature allows researchers to manipulate the PCR product without worrying about nonspecific amplification.

The polymerase has a 5′-3′ DNA polymerase and a 5′-3′ exonuclease activity, and catalyzes the non-template directed addition of an adenine residue to the 3′-end of both strands of DNA molecules, making it suitable for **TA cloning**. The proprietary polymerase/buffer formulation accommodates extended cycle numbers (45-50 cycles).

Protocol

The following protocol serves as a general guideline for routine PCR amplification. Optimal reaction conditions (annealing temperature and time, extension time, concentration of primers and template DNA) may vary and need to be optimized based on the template, primer composition, and PCR product length.

1. Prepare a 50 µL reaction mix according to the following guideline:

Reagent	50 µl PCR reaction	Final Concentration
10X PCR Buffer	5 µL	1x
Reversible 1-min Hot Start DNA Polymerase (2.5 Unit / µL)	0. 5 µL	1.25 unit
dNTP 10mM each	1 µL	200 µM
Forward Primer, 10 μM	2 µL	0.4 µM
Reverse Primer, 10 μM	2 µL	0.4 µM
Template DNA	< 1 µg	< 1 µg/reaction
Nuclease-Free Water	To a final volume of 50 µL	-

Note: For initial test, a final concentration of 0.4 µM for Forward and Reverse Primers can be used. To optimize the condition, test the range between 0.15 and 1.0 µM.
The composition of the 10X PCR buffer is: 100mM Tris-HCl, 500mM KCl, 15mM $MgCl_2$, PH 8.3 (at 25°C). Mg^{++} concentration may also be adjusted to achieve the best results (Mg^{++} concentration of 1.5-2.0 mM is optimal for Taq DNA polymerase. The final Mg^{++} concentration in the 1x PCR buffer is 1.5 mM, which is suitable for the amplification of most amplicons. However, Mg^{++} can be further optimized using MgCl2 included in the kit).

2. Program the PCR reaction conditions according to the following guideline:

Procedure	Temperature	Time	
Hot Start activation	95 °C	30 s - 1 min	
Denaturation	95 °C	10-15 s	30-40 cycles
Annealing	55-65 °C	30 s	
Extension	72 °C	30 s – 5 min	
Final extension	72 °C	2 min	

Note: To achieve the fastest turnaround, the Hot Start activation can be reduced to 30 seconds, and following denaturation step can be reduced to 10 seconds. Extension time should be adjusted according to the length of target PCR product size. The amplification speed of this Taq DNA polymerase is approximately 1 kb DNA / 60 seconds. The Annealing temperature should be adjusted based on primer and template composition to achieve the best results.

-- The end --