M5 Best Hotstar Taq DNA Polymerase User Manual

Product	Unit	Cat.#
M5 Best Hotstar Taq DNA Polymerase	500U	MF332-01
M5 Best Hotstar Taq DNA Polymerase	5x500U	MF332-05
M5 Best Hotstar Taq DNA Polymerase	10x500U	MF332-10
M5 Best Hotstar Taq DNA Polymerase	10000U	MF332-D

【Storage】

Store at -20℃ for up to 1 year and avoid freeze thawing. Stored at 4℃ for up to 3 months.

【Introduction】

Best Hotstar Taq DNA Polymerase is a kind of highly reliable hotstart polymerase. It has a 5′→3′ DNA polymerase and a 5′→3′ exonuclease activity. It also possesses 3′→5′ exonuclease (proofreading) activity. The polymerase has a higher amplification efficiency and lower mispairing rate compared to Taq DNA polymerase at routine PCR conditions. The polymerase is provided in an inactive state with no polymerase activity at ambient temperatures. This prevents the formation of misprimed products and primer dimers at ambient temperatures. Best Hotstar Taq Polymerase is activated by a 10 minutes, 95°C incubation step, which can easily be incorporated into existing thermal cycling programs. Due to the optimal buffer system, the target fragment can be amplified with high fidelity, high specificity, high amplification efficiency and high sensitivity. Best Hotstar Taq DNA Polymerase catalyzes the non-template directed addition of an adenine residue to the 3′-end of both strands of DNA molecules to make it suitable for TA cloning.

【COMPONENTS】

Cat. No.	**MF332-01**	**MF332-05**	**MF332-05**	**MF332-05**
Kit Size	500 U	5x500 U	10x500 U	10000U
Best Hotstar Taq DNA Polymerase, 5 U/μl	100 μl	5x100 μl	10x100 μl	2x1ml
5x Best Hotstar Taq PCR Buffer	1.9 ml	5x1.9ml	10x1.9ml	8x5ml

Note: 5x Best Hotstar Taq PCR Buffer contains 7.5 mM of Mg^{2+}.

【UNIT DEFINITION】

One unit is the amount of enzyme that will incorporate10 nmol of dNTP into acid-insoluble products in 30 minutes at 74℃ with activated salmon sperm DNA as the template-primer.

【APPLICATIONS】

1. PCR
2. RT-PCR
3. Multiplex PCR
4. Ideal for high specificity, high fidelity and high amplification efficiency PCR amplification

【KEY FEATURES】

1. High specificity, high fidelity and high amplification efficiency
2. Quick hot start: minimize the non-specific amplification products and shorten the reaction time

【PROTOCOL】

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of DNA polymerase, primers, $MgCl_2$, and template DNA) vary and need to be optimized according to the template, primer structure and target fragment size.

1. Prepare the reaction mix to 50 µl according to the following table.

Reagents	50 µl PCR reaction	Final Concentration
5x Best Hotstar Taq PCR Buffer	10 µl	1x
dNTP Mix, 2.5 mM each	4 µl	200 µM each
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
Best Hotstar Taq DNA Polymerase, 5 U/µl	0.5 µl	
RNase-Free Water	Up to 50 µl	

Note:
1) The recommended primer concentration for PCR is between 0.1-1.0 µM of each primer. The use of higher concentrations of primers can have for the higher amplification effect. Low primer concentration generally ensures cleaner product and lower background.

2) The recommended concentration range of Mg^{2+} should be 1.5-3 mM. 5x Super Hotstar Taq PCR Buffer in this kit contains 7.5 mM Mg^{2+} already and you can regulate its concentration according to the primers and templates to optimize the reaction system.

2. PCR reaction conditions

Procedure	Temperature	Time
Pre-denaturation	95˚C	10 min
		30-40 cycles
Denaturation	94˚C	30 s
Annealing	55-65˚C	30 s
Extension	72˚C	60 s
Final extension	72˚C	5 min

Note:
1) The common condition of annealing is about 5˚C below Tm for 30-60 seconds in PCR reactions. If extra bands are observed, higher annealing temperatures should be considered. The absence of product can indicate the need for a lower annealing temperature.

2) PCR extension time is depended on the target gene sequence and Best Hotstar Taq DNA polymerase is approximately 1 minute / kb DNA.

3) The number of PCR cycles will basically depend on the downstream application of the PCR product.

4) Best Hotstar Taq Polymerase requires to be activated by a 10 minutes, 95°C incubation step.

3. Analyze the PCR amplification products (ensure loading buffer is added) on an agarose gel.