Instructions for use

HS Q-Taq UP, 5 U/µl

100 µl (500 units)

DNA-free recombinant hot start Taq DNA polymerase, antibody blocked, for PCR amplifications, particularly of bacterial DNA

1. Description

HotStart version of the recombinant full-length form of the heat stable Taq DNA polymerase from the thermophilic bacterium *Thermus aquaticus* in storage buffer, tested on the absence of bacterial DNA.

For research use only. Not approved for use in clinical or *in vitro* diagnostics.

2. Applications

This ***Ultra-Pure HS Q-Taq*** DNA polymerase is recommended for use in PCR applications in general, or if bacterial DNA, or in RT-PCR, bacterial 16S rRNA shall be detected.
This DNA polymerase is appropriate for use in the amplification of DNA from genomic, viral, and plasmid templates, for high-yield PCR, for colony PCR and TA-cloning. The antibody-mediated blocking of the DNA polymerase is released only at the initial denaturation step, hence resulting in highly specific amplification of the target sequence without production of unwanted side products caused by unspecific primer annealing.
Ultra-Pure HS Q-Taq DNA polymerase is purified using a multiple-step process that minimizes contaminating bacterial DNA to a none detectable level. Each lot of the polymerase undergoes strict quality control testing in order to ensure the absence of detectable amounts of contaminating bacterial DNA.
Ultra-pure hot start Taq polymerase is able to amplify PCR products up to 3 kb with genomic DNA and up to at least 5 kb in size with Lambda DNA and is appropriate for use in the amplification of DNA from eukaryotic as well as prokaryotic templates. The ultra-pure hot start DNA polymerase possesses a 5´→ 3´ polymerase- as well as a 5´-3' exonuclease activity and generates a 3'dA (adenine)-overhang which may well be used for TA-cloning purposes.

3. Contents

Ultra-Pure HS Q-Taq DNA polymerase in storage buffer containing 50 % glycerol
PCR buffer (10x) with 20 mM $MgCl_2$.

Filled in colour coded tubes.

Reagent	Amount	Lid colour
Ultra-Pure HS Q-Taq DNA polymerase, 5 U/µl	1 tube, 100 µl	red
10x PCR buffer	1 tube, 1 ml	white

Ultra-Pure HS Q-Taq DNA polymerase and the 10x PCR buffer are both tested for the absence of residual bacterial DNA in a 16S rRNA-specific PCR assay. Reagents are also tested for the absence of contaminating human DNA.

4. Storage Buffer

50 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 0.5 % IGEPAL CA-630, 0.5 % Tween-20, 1 mM DTT, 50 % glycerol, mouse anti-Taq IgG

5. Enzyme activity

5 units/µl enzyme solution

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6. Unit definition

One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into an acid-insoluble DNA fraction in 30 minutes at 72 °C.

7. Suggested pipetting scheme

Due to the inhibition of polymerase activity at room temperature all reactions may be set up at room temperature. This will not result in an increase of unspecific product or primer-dimer formation.

Components	Apply for PCR reaction of 20 µl volume	Final concentration (recommended)
PCR buffer (10x) *	2 µl	1x
dNTP-Mix (2 mM)	2 µl	800 µM (200 µM each)
Forward primer (e.g. 5 pmol/µl)	variable (e.g. 1 µl)	0.1-0.5 µM
Reverse primer (e.g. 5 pmol/µl)	variable (e.g. 1 µl)	0.1-0.5 µM
Template DNA	variable	0.01-10 ng / reaction
Ultra-Pure HS Q-Taq polymerase (5 U/µl)	variable (i.e. 0.2 µl)	0.5-1.5 U
Sterile dest. water	adjust to 20 µl final volume	

* also see 3. Contents

8. Basic amplification protocol

Step	Time	Temperature
Initial denaturation	2 minutes	92-95 °C
25-35 cycles		
Denaturation	2-10 seconds	92-95 °C
Annealing	2-10 seconds	55-68 °C
Extension	variable, depends on the length of product	72 °C

9. Notes

IMPORTANT: For specific species-DNA detection and to avoid false positive results make sure your PCR buffer and other PCR reagents are free of DNA contaminations.

For maximum yield and specificity, annealing temperatures and annealing time as well as extension time and cycle numbers should be optimized for each template target and primer pair. Usually the optimal annealing temperature is 2-5 °C below the melting temperature of the primers. Recommended elongation time is 30-60 seconds per 1 kb of target. Elongation times of 30 seconds per 1 kb may be sufficient but longer elongation times may be necessary depending on the complexity of the template DNA. Product is not covered by pending or issued patents or may have certain limitations. To our best knowledge, this product does not provide any conflict with pending or issued patents.

10. Recommended $MgCl_2$ concentration

2 mM (final)

In case the $MgCl_2$ concentration has to be adjusted, use a separate $MgCl_2$ solution (10 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a $MgCl_2$ gradient in order to find the optimal concentration.

Pipetting scheme for additional $MgCl_2$

Final $MgCl_2$ conc. in mM	2.5	3	3.5	4
Add 10 mM $MgCl_2$ solution in following amounts to 20 µl reaction volume	1 µl	2 µl	3 µl	4 µl

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11. Storage conditions

Store the enzyme at -20°C. However, short term storage (few hours) of the enzyme may be done at ± 0°C (wet ice). The enzyme is also stable at room temperature for at least 3 days.
The buffer should be stored at -20°C, but may also be stored at +4 °C for several weeks.

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