

## Instructions for use

### Q-Taq UP, 5 U/ $\mu$ l

100  $\mu$ l (500 units)

#### DNA-free recombinant Taq DNA polymerase for PCR amplifications, particularly free from bacterial DNA

##### 1. Description

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 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.

**Ultra-Pure Q-Taq** 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 Taq 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 Q-Taq** DNA 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'  $\rightarrow$  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 Q-Taq** DNA polymerase in storage buffer containing 50 % glycerol.

PCR buffer (10x) with 20 mM MgCl<sub>2</sub>.

Filled in colour coded tubes.

Reagent	Amount	Lid colour
<b>Ultra-Pure Q-Taq</b> DNA polymerase, 5 U/ $\mu$ l	1 tube, 100 $\mu$ l	orange
10x PCR buffer	1 tube, 1 ml	white

**Ultra-Pure 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

##### 5. Enzyme activity

5 units/ $\mu$ l enzyme solution

## 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

At best prepare on ice:

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

\* also see 3. Contents

## 8. Basic amplification protocol

Step	Time	Temperature
Initial denaturation	2 minutes	92-95 °C
<b>25-35 cycles</b>		
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.

## 10. Recommended MgCl<sub>2</sub> concentration

2 mM (final)

In case the MgCl<sub>2</sub> concentration has to be adjusted, use a separate MgCl<sub>2</sub> solution (10 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a MgCl<sub>2</sub> gradient in order to find the optimal concentration.

### Pipetting scheme for additional MgCl<sub>2</sub>

Final MgCl <sub>2</sub> conc. in mM	2.5	3	3.5	4
Add 10 mM MgCl <sub>2</sub> solution in following amounts to 20 $\mu$ l reaction volume	1 $\mu$ l	2 $\mu$ l	3 $\mu$ l	4 $\mu$ l

### 11. Storage conditions

Store the enzyme at -20°C. However, short term storage (few hours) of the enzyme may be done at  $\pm 0^{\circ}\text{C}$  (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.

*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.*