Data sheet
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UltraSmarTaq DNA POLYMERASE

RESEARCH USE ONLY

Recombinant UltraSmarTaq DNA polymerase

(Deoxynucleosidetriphosphate: DNA Deoxynucleosidyltransferase E.C. 2.7.7.7.)

Cat.No.	Pack	Conc.
DUST-500	**500 Units**	**2,5 U/μl**
DUST-1000	**1000 Units**	**2,5 U/μl**

Stability:
Shelf life 18 months if store at -20°C

UNIT DEFINITION

One unit defined as the amount of enzyme that incorporates 10 nmoles of dNTP's into acid-insoluble form in 30 minutes at 74°C under assay conditions.

ASSOCIATED ACTIVITIES:

Endonuclease and exonuclease activities were not detectible after 2 and 1 hours incubation, respectively, of 1 μg lambda DNA and 0.22 μg of EcoR I digested lambda DNA, respectively, at 72°C in the presence of 15-20 units of **UltraSmarTaq** DNA polymerase.

STORAGE AND DILUTION BUFFER:

20mM Tris-HCL (pH 8.0); 100mM KCL; 0.1mM EDTA; 50% glycerol; 1% Tween-20, Stabilizer

AMPLIFICATION BUFFERS:

UltraSmarTaq DNA Polymerase is supplied with **2.5x Uni Buffer-1500**, containing 3.75 mM

SOURCE:

E.coli strain expressing the cloned **UlrtaSmarTaq** DNA Polymerase gene

DESCRIPTION

UltraSmarTaq DNA Polymerase is complex mixture of a thermostable 94 kDa **modified fused Taq DNA Polymerase special constriction** purified from E.coli strain expressing **Thermus aquatiqus** polymerase gene, **highly specific monoclonal antibodies and a fused, "Hi-Fi" ,antibodies –blocked enzyme.**

UltraSmarTaq, as hot-start enzyme, inactive under conditions of amplification reaction preparation (room temperature). It provides **improved specificity and high yield amplification, even with low copy number and complex DNA templates,** when compared to standard DNA polymerases**. UltraSmarTaq** can **eliminate amplification artifacts,** such as primer-dimer formation and miss priming.

An advantage of **UltraSmarTaq** is the absence of additional heating step for polymerase activation. Heat activation of enzyme occurs during the first denaturation step. An active complex of **UltraSmarTaq** dissociates automatically over **+70°C**, allowing activating DNA polymerase, preventing mispricing during amplification.

In the contrast to "regular" Taq-based enzymes, which inhibited by blood components modifications entered into **UltraSmarTaq** polymerase gene allowed to amplify DNA sequence **directly from whole blood**, stabilized with the commonly used agents (heparin, citrate or EDTA).

UltraSmarTaq DNA Polymerase possesses the 5'->3' DNA polymerase activity; 5'->3' exonuclease activity and generates A-tailed ends in the amplification products.

UltraSarTaq could be used in "fast-PCR" mode dramatically reducing time of PCR run – less than 40 min instead of 1,5-2 hours for the "regular" PCR.

UltraSmarTaq could be used in all "real-time" PCR applications including probe-based PCR (Taq-Man probes, beacons, etc.) or with intercollating dyes (SYBRGreen I / EvaGreen)

UltraSmarTaq Provides:

- Excellent performance across a wide range of "difficult" templates;
- Long range amplification of complex targets - > 5 kb from genomic DNA;
- High speed (1sec/100bp) elongation time for DNA template up to 2Kbp- reduce reaction times
- dUTP poisoning resistance
- "fast-PCR" mode amplification – 2 step PCR mode for most applications
- Excellent reproducibility in all types of "real-time" PCR
- **Excellent performance with GC-rich templates- Amplification of DNA templates up to 80% GC-content.**

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STORAGE CONDITIONS :
Store ***UltraSmarTaq*** DNA Polymerase at -20^{O}C for long term storage

SHIPPING CONDITIONS:
Should be shipped at ambient temperature. For long distance shipments preferably in **Blue Ice**

APPLICATIONS:
- Real-Time PCR, all types
- Multiplex amplification
- High sensitivity applications
- Low-copy number PCR
- Complex DNA template PCR
- "Anti-contamination" PCR
- "fast" PCR

General Protocol for amplification with UltraSmarTaq DNA polymerase

The optimal reaction conditions for **UltraSmarTaq** DNA polymerase differs from PCR protocols for standard (Taq-like) DNA polymerases. PCR conditions for **UltraSmarTaq DNA** Polymerase is similar in PCR conditions to **Phusion (ThermoScientific) ,Q5 polymerase (NEB)** or **iProof (Bio-Rad)** DNA polymerase, *e.g.* **UltraSmarTaq** polymerase works better at elevated denaturation and annealing temperatures.

PCR reactions should be set up even at room temperature, because of blocking of enzymatic activity by **specific monoclonal antibodies** at PCR setup conditions (usually RT).

UltraSmarTaq activates without pre-heating step, during "**Initial Denaturation**" PCR step. There is no need to enter additional heating step during PCR run.

Add and mix the following component:

Component	50μL reactions	25μL reactions	Final concentration
PCR grade Water	Up to 50 μL	Up to 25 μL	
2.5x Uni Buffer-1500*	20 μL	10 μL	1X
2,5 mM MIX dNTPs	4 μL	2 μL	0.2 mM each
Primers			0.1-0.4 μM each
Template DNA	optionally	optionally	
UltraSmarTaq (2.5 U/μl)*	1 μL	0.5 μL	0.02U/μL

We recommend using 50μl reaction for the PCR with UltraSmarTaq polymerase

Reaction Components

1.UltraSmarTaq Polymerase
- An optimal amount of enzyme in 50μl reaction is 2,5U. In some cases, it could be reduced up to 0.5U per reaction, depending on the length and complicity of amplified DNA sequence. For non-complex amplicons with the length less than 500bp and GC-content <60%, amount of UltraSmarTaq could be decreased up to 0.5-1.25U per 50μlreaction.

2. Buffer
- 2.5X Uni UltraSmarTaq buffers provides very high reproducibility across the wide range of amplification conditions, including "fast-PCR" (reduced time of PCR reaction). 2,5X Uni Buffer contains 1.5mM Mg^{2}+, as the final concentration. In some cases, we recommends to optimize Mg concentration in the range 1.5-4.5mM

3.dNTP's
- For most of applications, 200μM of each of dNTP's as final concentration is an optimal. It is not necessary to optimize dNTP's concentration. dUTP or other dUTP derivatives should be used replacing TTP in PCR reaction for "anti-contamination" PCR.

4. Primers
- Usually, 10-15 pmol of each specific primer in reaction is enough to get good PCR result. If you are using 2-step PCR with the whole blood as a template, it is better to use >= 20pmol of each primer.

5. PCR Additives
- UltraSmarTaq polymerase is compatible with the most of commonly used PCR additives for enhancing of high GC-content DNA templates (glycerol, beta in, DMSO and other).If one'll use any additives; take into account the changes of Tm of primers and DNA to correct annealing temperature.

6. GC-rich Templates Amplification
- For amplification of the GC-rich templates, it is not necessary to use any additives in reaction, because 2.5 X Uni Buffer-1500 contains already all reagents for the "fast" and reliable amplification. Amplification of templates with high GC-content (up to 80%) could be accomplish with UltraSmarTaq polymerase without any additional manipulations.

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UltraSmarTaq Polymerase Cycling Protocol:

Cycle step	2-step amplification		3-step amplification		Cycles
	T°C	Time	T°C	Time	
Initial Denaturation	**98°C**	**1-2 min**	**98°C**	**1-2min**	**1**
Denaturation	**98°C**	**2-5 S**	**98°C**	**2-5 S**	
Annealing	**-**	**-**	**58-72***	**10-30 S**	**30-40**
Extension	**67-72°C**	**15 S/Kb**	**72°C**	**5-15 S/Kb****	
Final extension	**72°C**	**0,5-1 min**	**72°C**	**1-3 min**	**1**
	4°C	**hold**	**4°C**	**hold**	

*Optimal Tm for the primer pair recommended as Tm of the lower primer, for the standard oligos <20nt.

For **UltraSmarTaq** polymerase Tm of the primers should be corrected, as +3-10°C, comparing with "regular" Taq-based enzymes PCR conditions.

1. Denaturation:

-1) for most applications time up to 1min of "Initial Denaturation" is quite enough. Empirically it could be reduce up to 30 seconds;

-2) For most applications, 2 sec at 98°C is enough for denaturation of the sample during PCR run.

NOTE: Do not use lower T denaturation, than 98°C; it can cause problems in PCR (nonspecific amplification, poor yield of PCR product, etc.)

2. Annealing/Extension:

For **UltraSmarTaq** polymerase "Annealing" and "Extension" steps should be combined if:

- Tm of both primers are not differs dramatically (<3°C);
- Tm of the primers are >65°C (optimal Tm for the primers lays between 65-70°C)
- If primers Tm is about 60-61°C for both primers ones can apply simple formula to determine starting Ta/e point - **(Tm of the lower primer +72°C)/2**. For most applications, it works fine.

-To determine an optimal Ta/e for better amplification results, run gradient amplification.

-To avoid non-specific band formation/smearing during amplification not exceed extension time of 30 seconds and use the highest ramp rate of amplificator (the ramp rate >4-5°C preferable)

**For non-complex DNA templates (plasmid DNA, phage DNA, BAC clone) extension time could be reduced up to 15 sec/Kb.

For complex DNA templates (human DNA) in amplification of the fragments longer than 2Kb strongly recommended to apply **Extension time** as 30 sec/Kb

For complex DNA templates (human DNA) in amplification of the fragments no longer, than 400-500bp (diagnostics value) combined Annelining/Extension times could be reduced up to 5 sec. per cycle

For the DNA templates with GC content less 65% and up to 500bp length, it is quite enough to set Extension time as 15 sec per cycle.

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