One-Fusion DNA Polymerase

Exceptional Polymerase: high speed proofreading even for long fragments

Features:
- Superior fidelity – about 50x improvement compared to Taq polymerase;
- Excellent performance across a wide range of “difficult” templates;
- Long range amplification of complex targets - > 12 kb from genomic DNA;
- High speed - reduce reaction times.
- dUTP poisoning resistance
- Resistance to blood containing DNA samples (up to 30% of blood)

Applications:
- High Fidelity (Hi-Fi) PCR
- Cloning
- “Hi–Fi” - LD PCR
- “anticontaminated” PCR

Description:
One-Fusion DNA Polymerase is a unique artificial enzyme created on the basis of intellectual protein design planning by genetic engineering technique. The enzyme possess high fidelity feature. The processivity of the enzyme is very high, so the combination of processivity with fidelity results in dramatically increased yield of PCR products, very high sensitivity of PCR tests, ability to amplify “difficult” templates.
This dramatic increase in processivity results not only in shorter extension times, but in more robust amplification and the ability to amplify long templates really fast.
One-Fusion DNA Polymerase possesses the 5'->3' DNA polymerase activity, 3'->5' exonuclease activity and temperature-depended strand-displacement activity and generates blunt ends in the amplification products.

Unit definition:
One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP's into acid-insoluble form in 30 minutes at 75°C under assay conditions: 25 mM TAPS-HCl, pH 9.0 (at 25°C), 100 mM KCl, 1.5 mM $MgCl_2$, 1 mM Beta-mercaptoethanol, 200 µM each dNTP, and 10 µg activated calf thymus DNA in 50 µl.

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 One-Fusion DNA polymerase.

Storage buffer:
20mM Tris-HCl, pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM DTT, 50% Glycerol,0.5% Tween 20, stabilizers

Reaction buffers provided:
2,5X Unibuffer: containing 3,75 $MgCl_2$, in the provided 2,5X concentration

Storage: store @ - 20°C

Transport: can be send at ambient temperature, preferable is transport “on ice”

General protocol:
The optimal reaction conditions for One-Fusion DNA Polymerase may differ from PCR protocols for standard (Taq-like) DNA polymerases.
PCR conditions for One-Fusion DNA Polymerase is more similar in PCR conditions to “Fusion-like” DNA polymerases, e.g. the enzyme works better at elevated denaturation and annealing temperatures.

PCR reactions should be set up on ice. Prepare a master mix for the appropriate number of samples to be amplified.

Note!

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It is critical that the One-Fusion DNA polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3'->5' exonuclease activity that can degrade primers in the absence of dNTPs.

Component	50µl reactions	25µl reactions	Final concentration
PCR grade Water	Up to 50 µl	Up to 25 µl	
2.5x UNI Buffer	Up to 20µl	Up to 10 µl	1X
10 mM MIX dNTPs	1 µl	0.5 µl	0.2 mM each
Primers			0.3-0.5 µM each
Template DNA	optionally	Optionally	
One-Fusion polymerase (2 U/µ)*	0,5-1 µl	0,25-0,5 µl	Up to 0.02U/µL

* both buffers results in 1.5mM Mg2+, as the final concentration.
In some cases we recommend to optimize Mg concentration in the range 1.5-2.5mM
We recommend to use 50µl reaction for the PCR with One-Fusion polymerase

Cycling:

Cycle step	2-step amplification		3-step amplification		Cycles
	T°C	Time	T°C	Time	
Initial Denaturation	**98°C**	**2-5 min**	**98°C**	**2-5 min**	**1**
Denaturation	**98°C**	**5-10 S**	**98°C**	**5-10 S**	
Annealing	**-**	**-**	**55-72***	**10-30 S**	**25-35**
Extension	**72°C**	**15-30 S/Kb**	**72°C**	**15-30 S/Kb****	
Final extension	**72°C**	**1-3 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 One-Fusion polymerase Tm of the primers should be corrected, as +3-5°C, comparing with Taq-based PCR conditions.**

****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) strongly recommended to apply extension time as 30 sec/Kb

Ordering information:

Cat.-no	Description	Amount
S450	DNA Polymerase	200 units
S455	DNA Polymerase	1000 units