TOO™ LONG DNA Polymerase Kit

Ordering Info

TBK0045, 20 U (5U/ µL)(sample)

TBK0046, 500 U (5U/ µL)

TBK0047, 1.000 U (5U/ µL)

Description

TOO™ LONG DNA Polymerase Kit is a convenient kit that includes TOO™ LONG DNA polymerase and highly pure High-Q™ dNTPs to enable the amplification of targets since 5 kb to 20 kb.

TOO™ LONG DNA polymerase is a blended enzyme preparation which combines a polymerase with 3'-5' exonuclease activity with a polymerase lacking 3'-5' exonuclease activity. PCR amplification generates a mixture of A-overhang-ended (predominantly) and blunt-ended PCR products.

Features

- **Efficient long targets amplifications (5-20 kb).**
- PCR fragments **suitable to be cloning in TA-vectors or blunt vectors.**
- **Increased yield and fidelity** of PCR products.
- Error Rate 5.6 x10^{-6} errors/bp per cycle[1].

Applications

- Suitable as a direct replacement for ordinary STOUT™ Recombinant Taq DNA Polymerase in most applications.
- Generation of PCR fragments for TA or blunt cloning.
- Sequencing.
- DNA Labeling.

[1] Nucleic Acids Research (1996), 24(18), 3546–3551.

Kit Components

Components	TBK0046	TBK0047
TOO™ LONG DNA polymerase (5 U/ µL)	100 µL	200 µL
TOO™ LONG PCR Buffer (10x)	1.5 mL	2 x 1.5 mL
MgCl2 25 mM	1.5 mL	2 x 1.5 mL
High-Q™ dNTPs 10 mM $_{TOTAL}$	1 mL	2 x 1 mL
DMSO 100%	50 µL	50 µL

Order Info Kit Components: *TOO™ LONG PCR Buffer 10x (TBB0312) | MgCl2 25 mM (TBR0215) | High-Q™ dNTPs 10 mM $_{TOTAL}$ (TBR0209) | DMSO (TBR0260).*

Storage

Store at -20°C. Shipped in blue ice.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into an acid-insoluble form in 30 minutes at 70°C.

Quality Control

Functionally tested.

Material required (not supplied)

- PCR Tubes
- PCR Grade Water, nuclease free (TBB0303)
- Specific Primers

PROTOCOL

1. Thawing all components on ice. Vortex and centrifugate them.

2. On ice, prepare a mix of the following components, considering the number of samples plus two extra reactions.

Reaction Components	Final Concentration	Volume	Volume
TOO™ LONG PCR Buffer 10x	1 x	2 µL	5 µL
MgCl2 25 mM	2.5 mM	2 µL	5 µL
High-Q™ dNTPs Mix 10 mM TOTAL	0.8 mM	1.6 µL	4 µL
Forward Primer 15 µM (15 pmol/ µL)	0.4 µM*	0.5 µL	1.3 µL
Reverse Primer 15 µM (15 pmol/ µL)	0.4 µM*	0.5 µL	1.3 µL
TOO™ LONG DNA polymerase (5 U/ µL)	0.05 U/ µL	0.2 µL	0.5 µL
Water, molecular biology grade		up 20 µL**	up 50 µL**
DNA template (add in step 4)		**	**
Final Volume		**20 µL**	**50 µL**

** Optimal between 0.1-1 µM* *** consider volume of template to be added in step 4.*
DMSO is recommended for GC-rich amplicons to a final concentration of 1-10%.

3. Distribute the mix prepared in each PCR tube or well.

4. Add in each tube the DNA sample (plasmid DNA: 10-75 ng; gDNA: 100-500 ng). Mix well.

5. Set up thermocycler:

Process	Cycles	Temperature	Time
Initial denaturation	1 x	94 °C	2:00
Denaturation	25 - 30 x	94 °C	0:15
Annealing		Tm	0:20
Extension		68 °C	1:00 per kb
Final Extension	1 x	68 °C	10:00
Conservation	1 x	4 °C	∞