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 TOO™ LONG PCR Buffer (10x) 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.

Kit Components

Components	TBK0046	TBK0047
TOO™ LONG DNA polymerase (5 U/ µL)	100 µL	2x100 µL
TOO™ LONG PCR Buffer (10x)	2x1 mL	4 x 1 mL

Order Info Kit Components: TOO™ LONG PCR Buffer 5x (TBB0312)

Storage

Store at -20°C. Shipped in blue ice.

TOO™ LONG PCR Buffer (10x)

TOO™ LONG PCR Buffer (10x) contains Mg2+ (2 mM at the final, 1x, reaction concentration). Mix the Reaction Buffer solution thoroughly after thawing.

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 72°C.

Quality Control

Functionally tested.

Material required (not supplied)

- PCR Tubes
- PCR Grade Water, nuclease free (TBB0303)
- dNTPs Mix
- 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
TOO™ LONG PCR Buffer 10x	1 x	5 µL
Forward Primer 15 µM (15 pmol/ µL)	0.4 µM	1.3 µL
Reverse Primer 15 µM (15 pmol/ µL)	0.4 µM	1.3 µL
dNTPs Mix	0.3 (0.2-0.5) mM	
TOO™ LONG DNA polymerase (5 U/ µL)	0.1 U/ µL	1 µL
Water, molecular biology grade		up 50 µL
DNA template (add in step 4)		**
Final Volume		**50 µL**

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	0:20
Annealing		Tm	0:30
Extension		68 °C	1:00 per kb
Final Extension	1 x	68 °C	1:50 per kb
Conservation	1 x	4 °C	∞