TOO™ LONG DNA Polymerase Master Mix (2x)

Ordering info

TBK0048, 5 reactions (sample)

TBK0049, 100 reactions

TBK0050, 200 reactions

Description

TOO™ LONG DNA Polymerase Master Mix (2x) is an optimized formulation to enable the amplification of targets since 5 kb to 20 kb. It is based in a blended enzyme preparation which combine 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).
- Ready to use, avoiding mistakes in PCR reaction preparation.
- 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	TBK0049	TBK0050
TOO™ LONG DNA Polymerase Master Mix (2x)	2 x 1.25 mL	4 x 1.25 mL
PCR Grade Water, nuclease free	2 x 2 mL	4 x 2 mL

Order Info Kit Components: *PCR Grade Water, nuclease free (TBB0303).*

Storage

Store at -20°C. Shipped in blue ice.

Quality Control

Functionally tested.

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.

Material required (not supplied)

- PCR Tubes.
- Specific primers.

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

PROTOCOL

I. PREPARING REACTIONS

1. Gently vortex and briefly centrifuge all solutions after thawing.

2. Place a tube on ice and add the following components for each 50 µL reaction. Prepare sufficient master mix for the number of reactions. Consider one or two extras:

Components	Volume	Final Concentration
TOO™ LONG DNA Polymerase Master Mix (2x)	25 µL	1x
Forward primer 15 µM (15 pmol/µL)	1.3 µL	0.4 µM*
Reverse primer 15 µM (15 pmol/µL)	1.3 µL	0,4 µM*
PCR Grade Water, nuclease-free	up 50 µL	
Template DNA		**
Final Volume	**50 µL**	

** Optimal between 0.1-1 µM.*

*** Optimal amounts of template DNA are 10-30 ng for both plasmid and phage DNA, and 0.3-1 µg for genomic DNA.*

3. Aliquot the master mix into individual PCR tubes.

4. Gently vortex and spin down the samples. Add template DNA.

II. PCR SETUP

1. Perform PCR using recommended thermal cycling conditions:

Step	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	∞

2. Store the samples at -20°C until use.