STOUT™ FAST DNA Polymerase Kit

Ordering Info

TBK1011, 500 U (5U/ µL)

TBK1012, 2.500 U (5U/ µL)

Description

STOUT™ FAST DNA Polymerase Kit is a recombinant polymerase with a fast polymerization range (4-8 kb/ min) generating consistent amplification results. The enzyme has 5´→ 3´ polymerase activity and 5´→ 3´ exonuclease activity while 3´→ 5´ exonuclease activity is absent. It is an extraordinary enzyme for routine PCR of genotyping or screening.

Features

- Fast Amplification of PCR targets up to 5 kb.
- High Extension Rate, 2 seconds/ kb for targets < 1 kb.
- Addition without template of 3´adenine at the end of PCR fragment.
- Non-proofreading polymerase.
- Immediate Activation.

Applications

- Fast PCR.
- Generation of PCR fragments for TA cloning.
- Genotyping.
- Screening by PCR.

Kit Components

Components	TBK1011	TBK1012
STOUT™ FAST DNA Polymerase (5 U/ µL)	100 µL	500 µL
FAST Reaction Buffer 5x*	4 x 1 mL	20 mL

**FAST Reaction Buffer 5x Includes 15 mM $MgCl_2$ and 5 mM $_{TOTAL}$ dNTPs.*

Order Info Kit Components: *STOUT™ FAST DNA Polymerase (TBK1011-1) | FAST Reaction Buffer 5x (TBK1011-2).*

Storage

Store at -20°C. Shipped in blue ice.

Quality Control

Functionally tested in a 1 kb PCR amplification (GC 52%).

Material required (not supplied)

- PCR Grade Water (TBB0303)
- PCR Tubes
- Specific Primers

Also available:

STOUT™ Recombinant Taq DNA Polymerase Master Mix (2x) (TBK0028, TBK0029)

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
FAST Reaction Buffer 5x	1 x	5 µL	10 µL
Forward Primer (10 pmol/ µL)	0.4 µM	1 µL	2 µL
Reverse Primer (10 pmol/ µL)	0.4 µM	1 µL	2 µL
STOUT™ FAST DNA Polymerase (5 U/ µL)	0.5 U/ µL*	0.1 µL	0.2 µL
Water, molecular biology grade		up 25 µL*	up 50 µL*
DNA template (add in step 4)		**	**
Final Volume		**25 µL**	**50 µL**

** for complex targets (high GC content, poorly purified samples, etc), increase the enzyme concentration.*
*** consider volume of template to be added in step 4.*

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

4. Add in each tube the DNA sample (cDNA: < 50 ng; gDNA: < 250 ng). Mix well.

5. Set up thermocycler:

Process	Cycles	Temperature	Time
Initial denaturation	1 x	94 °C	1:00
Denaturation	25-40 x	94 °C	0:15
Annealing		Tm	0:15
Extension		72 °C	0:02 per kb (< 1 kb) 0:15 per kb (> 1 kb)
Final Extension	1 x	72 °C	3:00
Conservation	1 x	4 °C	∞