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Manual

WALK DNA polymerase

Pwo DNA polymerase with reaction buffer. Concentration 1 U/µl.

catalog #	size
1002-200	200 U
1002-1000	1000 U

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Advantages

- Highest fidelity thermostable *Pwo* DNA polymerase (*Pyrococcus woesei)*
- Proofreading
- Highest purity enzyme
- Recommended for cloning - generates blunt-ended DNA

Description

WALK DNA polymerase is recombinant thermophilic DNA polymerase purified from *E.coli* stream carrying a plasmid with a cloned gene encoding a DNA polymerase from *Pyrococcus woesei*.
Enzyme catalysis incorporation of deoxynucleotides to 3' end of dsDNA at temperature 70-80 °C and presence of Mg^{2+} ions. Enzyme generates blunt-ended ds DNA fragments.
Pwo DNA polymerase possesses 3'-5' exonuclease activity (proofreading) responsible for high fidelity of the enzyme, but lacks 5'-3' exonuclease activity.

Contents

	1002-200	**1002-1000**	**storage**
WALK polymerase	200 U (1 U/µl)	1000 U (1 U/µl)	-20 °C
storage buffer: 10 mM KCl, 20 mM Tris-HCl pH 8.7, 0,1 mM EDTA, stabilizers, 50% glicerol (v/v).			
WALK reaction buffer	1 x 1.5 ml	4 x 1.5 ml	-20 °C
10x PCR reaction buffer: 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl, pH 8.5, 20 mM $MgSO_4$, 1% Triton X-100.			

Notes

- Before using, thoroughly thaw and gently mix by inverting the tubes.

Example PCR protocol

1. Thaw all components on ice, gently mix by inverting the tubes and briefly centrifuge. Place the tubes on ice again.
2. Place PCR tubes on ice and add:

	PCR reaction volume
component	**50 µl**
WALK reaction buffer	5 µl
dNTP Mix (10 mM)	200-250 µM (1-1.25 µl)
Starter 1	0,1-0,5 µM
Starter 2	0,1-0,5 µM
WALK polymerase	1-2 U
DNA template	10 pg -1 µg
Sterile water	up to 50 µl

3. Gently mix the samples and briefly centrifuge.
4. Place the tubes in the thermocycler and start the PCR programme.

   An example amplification profile for products up to 1000 bp:

step	**temperature**	**time**
Initial denaturation	94 °C	1-5 min
25-45 cycles	94 °C 50-68 °C 72 °C	30-60 s 30-60 s 2 min
Final incubation	72 °C	5-10 min

   For longer fragments we recommend doubling the elongation time in relation to the time used for *Taq* DNA polymerase (RUN DNA polymerase).
5. PCR products store in a refrigerator or freezer until later use.

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