Instructions for use

ROTI®Pol ProofRead

Recombinant Pfu DNA proof-reading polymerase for high fidelity PCR amplifications
9344

1. Description

Recombinant version of the heat stable Pfu DNA polymerase from the extreme thermophilic archae bacterium *Pyrococcus furiosus* in storage buffer, plus additional 10x concentrated PCR-ProofRead reaction buffer.

For research use only. Not approved for use in clinical or *in vitro* diagnostics.

2. Applications

This polymerase set ROTI®Pol ProofRead is the optimal choice for all high fidelity PCR assays, as being performed, for instance, prior to cloning or sequencing, in cycle sequencing or in site-directed mutagenesis reactions. In combination with the attached specially adjusted buffer, the ProofRead polymerase delivers sequence identical PCR amplificates of good yield with a wide range of PCR templates. Additionally, ROTI®Pol ProofRead is well suited for amplification of target sequences of high GC content, or with complex secondary structures.
ROTI®Pol ProofRead is able to amplify PCR products up to 3 kb with genomic DNA and is appropriate for use in the amplification of a broad variety of template DNAs. The ProofRead DNA polymerase included in the set replicates DNA 5´→ 3´ at 72 °C to 75 °C under presence of magnesium ions. Furthermore, the ProofRead-DNA polymerase possesses a 5´→ 3´ (proof reading) exonuclease activity, rapidly substituting misincorporated bases during polymerization, and thus being responsible for the high sequence fidelity.
ROTI®Pol ProofRead generated DNA fragments are *blunt-ended*.

3. Set contents

ProofRead polymerase (Art. No. 0560) in storage buffer containing 50 % glycerol
PCR ProofRead buffer (10x) (Art. No. 0553) with 20 mM $MgSO_4$
Filled in colour coded tubes.
Contents of this set may not be bought separately.

Reagent	Lid colour	9344.1	9344.2
ProofRead polymerase	orange	1 tube	5 tubes
PCR ProofRead buffer (10x)	brown	1 tube	5 tubes

The use of the colourless PCR reaction buffer is adequate for all general PCR applications.

4. Storage Buffer

20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 0.1 % IGEPAL CA-630, 0.1 % Tween-20, 0.05 % CHAPS, 1 mM DTT, 50 % glycerol

5. Enzyme activity

5 units/µl enzyme solution

6. Unit definition

One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into an acid-insoluble DNA fraction in 30 minutes at 72 °C.

7. Suggested pipetting scheme

Prepare on ice:

Components	Apply for PCR reaction of 20 µl volume	Final concentration (recommended)
PCR ProofRead buffer (10x)	2 µl	1x
dNTP-Mix (2 mM)	2 µl	800 µM (200 µM each)
Forward primer (e.g. 5 pmol/µl)	variable (e.g. 1 µl)	0.1-0.5 µM
Reverse primer (e.g. 5 pmol/µl)	variable (e.g. 1 µl)	0.1-0.5 µM
Template DNA	variable	0.01-10 ng / reaction
ProofRead Polymerase (5 U/µl)	variable (i.e. 0.2 µl)	0.5-1.5 U
Sterile dest. water	adjust to 20 µl final volume	

8. Basic amplification protocol

Step	Time	Temperature
Initial denaturation	2 minutes	95 °C
25-35 cycles		
Denaturation	2-10 seconds	95 °C
Annealing	2-10 seconds	55-68 °C
Extension	variable, depends on the length of product	72 °C

9. Notes

For maximum yield and specificity, annealing temperatures and annealing time as well as extension time and cycle numbers should be optimised for each template target and primer pair. Usually the optimal annealing temperature is 2-5 °C below the melting temperature of the primers. Recommended elongation time is 60 secs. per 1 kb of target.
ROTI®Pol ProofRead (Pfu polymerase) has a slower processivity than standard Taq DNA polymerases. For a start, double the elongation time usualy calculated for the use with ROTI®Pol TaqS or other Taq DNA polymerases. Elongation times may also depend on the complexity of template DNA.

Product is not covered by pending or issued patents or may have certain limitations. To our best knowledge, however, this product does not provide any conflict with pending or issued patents.

10. Recommended $MgSO_4$ concentration

2-4 mM
Generally, 2 mM $MgSO_4$ is suitable for polymerization of most assays. However, in those cases in which the $MgSO_4$ concentration has to be adjusted, use a separate $MgSO_4$ solution (100 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a $MgSO_4$ gradient in order to find the optimal concentration.

Pipetting scheme for additional $MgSO_4$

Final $MgSO_4$ conc. in mM	2.5	3	3.5	4
Add 100 mM $MgSO_4$ solution in following amounts to 20 µl reaction volume	0.1 µl	0.2 µl	0.3 µl	0.4 µl

11. Storage conditions

Store at -20 °C. The buffer may be stored at -4 °C.
Infrequent short term storage (few hours) of the enzyme may be done at +4 °C.

12. Additionally recommended products

For our Thermal cyclers please contact us under 0721 / 5606 - 0

ROTI®Mix PCR 3 (10 mM per dNTP dATP, dTTP, dGTP, dCTP)	Art. No. L785
ROTI®Mix PCR 3 (pH 7) (10 mM per dNTP dATP, dTTP, dGTP, dCTP)	Art. No. 0179
dNTP-Set 1 (≥99 %, 100 mM pure solutions dATP, dTTP, dGTP, dCTP)	Art. No. K039
dNTP-Set 1 (pH 7) (≥99 %, 100 mM pure solutions dATP, dTTP, dGTP, dCTP)	Art. No. 0178
Mineral oil (for or overlaying PCR and other enzymatic reactions)	Art. No. HP50
ROTI®Nucleic acid-free (ready-to-use solution for removal of DNA from surfaces)	Art. No. HP69
ROTI®Nucleic acid-free eXtra (ready-to-use, gentle solution for DNA removal)	Art. No. 1312
DNA AWAY® (ready-to-use solution for removal of DNA from surfaces)	Art. No. X996

Please note our full range of DNA polymerases and MasterMixes:

ROTI®Pol TaqS	Art. No. 9223
ROTI®Pol TaqS Mix	Art. No. 9239
ROTI®Pol TaqS Red-Mix	Art. No. 9241
ROTI®Pol Hot-TaqS	Art. No. 9245
ROTI®Pol Hot-TaqS Mix	Art. No. 9248
ROTI®Pol Hot-TaqS Red-Mix	Art. No. 9256
ROTI®Pol TaqHY	Art. No. 9345
ROTI®Pol Hot-TaqHY	Art. No. 9346
ROTI®Pol ProofRead	Art. No. 9344
ROTI®Pol TaqUltra	Art. No. 9347
ROTI®Pol Hot-TaqUltra	Art. No. 9350

ROTI®Pol ProofRead	200 U	incl. PCR Buffer	**9344.1**
	1.000 U	incl. PCR Buffer	**9344.2**

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