Instructions for use

ROTI®Pol Hot-TaqS

Recombinant hot start Taq DNA polymerase, antibody blocked
9245

1. Description

Hot start version of the recombinant heat stable Taq DNA polymerase from the thermophilic bacterium *Thermus aquaticus* in storage buffer, plus additional 10x concentrated PCR reaction buffer and 10x concentrated PCR reaction buffer with red gel loading dye.
For research use only. Not approved for use in clinical or *in vitro* diagnostics.

2. Applications

This polymerase set ROTI®Pol Hot-TaqS is outstandingly suitable for all Taq-based cycling protocols, in which particularly specific amplification is the main focus - as being performed in, for instance, prior to cloning or sequencing processes, in cycle sequencing, and in similar assays. In combination with our unique buffers, the Hot-TaqS polymerase delivers highly specific PCR amplification of good yield with a wide range of PCR templates. The antibody-mediated blocking of the DNA polymerase is released only at the initial denaturation step, hence resulting in highly specific amplification of the target sequence without production of unwanted side products caused by unspecific primer annealing.
ROTI®Pol Hot-TaqS is able to amplify PCR products up to 3 kb with genomic DNA and up to at least 5 kb in size with Lambda DNA and is appropriate for use in the amplification of DNA from genomic, viral, and plasmid templates. The Hot-TaqS DNA polymerase included in the set possesses a 5´→ 3´ polymerase- as well as a 5´-flap endonuclease activity, and generates a 3'dA (adenine)-overhang which may well be used for TA-cloning purposes.

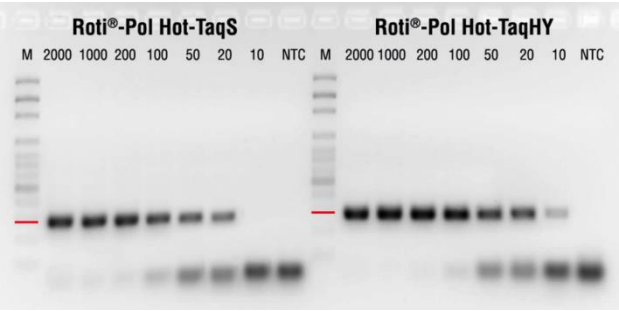

Figure:
Sensitivity assay using ROTI®Pol Hot-TaqS and Hot-TaqHY,
1 U/reaction each (20 µl).
300 bp β-Actin fragment, 40 cycles.
Template: 10 to 2.000 pg human gDNA.
Gel loading 10 µl each.
M: 100 bp-DNA Ladder extended.
NTC: no template control.

3. Set contents

Hot-TaqS polymerase (Art. No. 0521) in storage buffer containing 50 % glycerol
PCR buffer (10x) (Art. No. 0511) with 20 mM $MgCl_2$
PCR buffer red (10x) (Art. No. 0527) with 20 mM $MgCl_2$ and 0.1 % cresol red (ready-to-load)
Filled in colour coded tubes.
Contents of this set may not be bought separately.

Reagent	Lid colour	9245.1	9245.2
Hot-TaqS polymerase	red	1 tube	5 tubes
PCR buffer (10x)	blue	1 tube	5 tubes
PCR buffer red (10x)	violet	1 tube	5 tubes

The PCR buffer red (10x) contains a red dye which functions as a loading dye. The buffer has sufficient density for direct loading of PCR reactions onto an agarose gel for PCR product analysis. The red dye migrates in a 1% agarose gel at the same rate as a ~1kb DNA fragment. The dye turns yellow at an acidic pH, for instance during Southern-Blotting. The use of the colourless PCR reaction buffer is adequate for all general PCR applications and is particularly recommended when direct fluorescence or absorbance readings are required.

4. Storage Buffer

50 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 0.5 % Nonidet-P40, 0.5 % Tween-20, 1 mM DTT, 50 % glycerol, mouse anti-Taq IgG

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

Due to the inhibition of polymerase activity at room temperature all reactions may be set up at room temperature. This will not result in an increase of unspecific product or primer-dimer formation.

Components	Apply for PCR reaction of 20 µl volume	Final concentration (recommended)
PCR 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
Hot-TaqS 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	92-95 °C
25-35 cycles		
Denaturation	2-10 seconds	92-95 °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 30-60 secs. per 1 kb of target. Elongation times of 30 secs. per 1 kb may be sufficient but longer elongation times may be necessary depending on the complexity of the 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 $MgCl_2$ concentration

2-4 mM

In case the $MgCl_2$ concentration has to be adjusted, use a separate $MgCl_2$ solution (100 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a $MgCl_2$ gradient in order to find the optimal concentration.

Pipetting scheme for additional $MgCl_2$

Final $MgCl_2$ conc. in mM	2.5	3	3.5	4
Add 100 mM $MgCl_2$ 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
PCR water for molecular biology, sterile, ready-to-use	Art. No. 1HPE
Magnesium chloride solution 25 mM, for PCR, for molecular biology	Art. No. 1HY7
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 TaqHY Mix	Art. No. 1K33
ROTI®Pol TaqHY Red-Mix	Art. No. 1K34
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 Hot-TaqS	200 U	incl. PCR Buffers	9245.1
	1.000 U	incl. PCR Buffers	9245.2

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