Instructions for use

ROTI®Pol Hot-TaqUltra

DNA-free recombinant hot start Taq DNA polymerase, antibody blocked, for PCR amplifications, particularly of bacterial DNA
9350

1. Description

Hot start version of the recombinant full-length form of the heat stable Taq DNA polymerase from the thermophilic bacterium *Thermus aquaticus* in storage buffer, and tested on the absence of bacterial DNA.
For research use only. Not approved for use in clinical or *in vitro* diagnostics.

2. Applications

This ROTI®Pol Hot-TaqUltra is recommended for use in PCR applications in general, or if bacterial DNA, or in RT-PCR, bacterial 16S rRNA shall be detected.
The polymerase is appropriate for use in the amplification of DNA from genomic, viral, and plasmid templates, for high-yield PCR, for colony PCR and TA-cloning. 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-TaqUltra is purified using a multiple-step process that minimises contaminating bacterial DNA to a none detectable level. Each lot of the polymerase undergoes strict quality control testing in order to ensure the absence of detectable amounts of contaminating bacterial DNA.
ROTI®Pol Hot-TaqUltra 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 eukaryotic as well as prokaryotic templates. The Hot-TaqUltra DNA polymerase possesses a 5´→ 3´ polymerase- as well as a 5´-3' exonuclease activity, and generates a 3'dA (adenine)-overhang which may well be used for TA-cloning purposes.

3. Content

ROTI®Pol Hot-TaqUltra polymerase (Art. No. 9350) in storage buffer containing 50 % glycerol
Filled in red-capped tubes.

Reagent	Lid colour	9350.1	9350.2
Hot-TaqUltra polymerase	red	1 tube	5 tubes

This DNA polymerase is provided without reaction buffer.

Since the ROTI®Pol Hot-TaqUltra is an antibody-blocked, particularly high purified version of the TaqS polymerase, the buffer included in the ROTI®Pol TaqS and Hot-TaqS products (as well as in the ROTI®Pol TaqHY and Hot-TaqHY products) is perfectly suited for use as reaction buffer for the Hot-TaqUltra as well (see also 12. Additionally recommended products).
In addition to our specifically optimised reaction buffers, general Tris-based Taq polymerase reaction buffers as, for instance, given in *Molecular Cloning* (Sambrook and Russell, Cold Spring Harbour Laboratory Press), work well for the ROTI®Pol Hot-TaqUltra enzyme. We recommend use of 20 mM $MgCl_2$ and 0.1 % Tween®-20.

Please note, however, that the batches of our reaction buffers *have not been tested for presence of DNA or residual bacteria* which might lead to false-positive results. For each buffer that shall be used for the TaqUltra enzyme, either taken from a ROTI®Pol product or self-made, we recommend to do preliminary studies in order to ensure the freeness of DNA functioning as target for the particular primer pairs to be used.

4. Storage Buffer

50 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 0.5 % IGEPAL CA-630, 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
ROTI®Pol Hot-TaqUltra polymerase (5 U/µl)	variable (i.e. 0.2 µl)	0.5-1.5 U
Sterile dest. water	adjust to 20 µl final volume	

*also see 3. Content

8. Basic amplification protocol

Step	Time	Temperature
Initial denaturation	2 minutes	92-95 °C
25-40 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

IMPORTANT: For specific species-DNA detection and to avoid false positive results make sure your PCR buffer and other PCR reagents are free of DNA contaminations.

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. 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-TaqUltra	200 U	**9350.1**
	1.000 U	**9350.2**

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