# Instructions for use

# ROTI®Pol Hot-TaqHY

**Modified recombinant hot start Taq DNA polymerase, antibody blocked, for high yield PCR amplifications**
**9346**

## 1. Description

Modified version of the recombinant hot start 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-TaqHY is superior for all sophisticated Taq-based cycling protocols, in which both, particularly specific amplification as well as very high yields shall be obtained, or if highly specific amplifications shall be performed using particularly short cycle times. Thus, ROTI®Pol Hot-TaqHY is the perfect choice prior to cloning or sequencing processes, for cycle sequencing and for colony PCR, and it may also well be used in qPCR- and multiplex PCR applications.
Combining the benefits of both techniques, the Hot-TaqHY DNA polymerase has been designed by increasing the PCR sequence specificity of a quick and high yield modified Taq polymerase by antibody mediated inhibition. In combination with our unique buffers, the Hot-TaqHY polymerase delivers highly specific PCR amplification of superior yield with a wide range of PCR templates.
The blocked Taq polymerase is getting active only after the initial denaturation step, resulting in highly specific amplification of the target sequence without production of unwanted side products due to unspecific primer annealing. In parallel, the modification of the DNA polymerase results in significantly enhanced amplicon yields, with the benefit of shorter elongation times needed.
ROTI®Pol Hot-TaqHY 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 a wide range of even complex templates. The Hot-TaqHY 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.

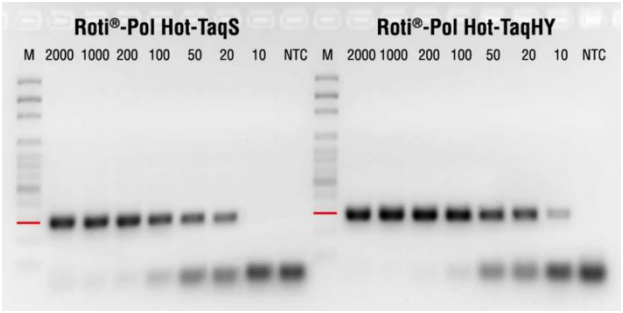


**Figure:**
Sensitivity assay using ROTI®Pol Hot-TaqS and Hot-TaqHY, 1 U/reaction each (20 µl).
300 bp β-Actin fragment, 40 cycles.
Template: 10 to 2.000 pg human gDNA.
Gel loading 10 µl each.
M: 100 bp-DNA Ladder extended.
NTC: no template control.

## 3. Set contents

Hot-TaqHY polymerase (Art. No. 0535) 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 | 9346.1 | 9346.2 |
|---|---|---|---|
| **Hot-TaqHY 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-TaqHY 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

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|---|---|
| **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:

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|---|---|
| **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 |

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|---|---|---|---|
| **ROTI®Pol Hot-TaqHY** | 200 U | incl. PCR Buffers | 9346.1 |
| | 1.000 U | incl. PCR Buffers | 9346.2 |

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