

Instructions for use



ROTI®Pol TaqHY Mix (2x)

Modified recombinant Taq DNA polymerase master mix for high yield PCR amplifications

1K33

1. Description

Optimised pre-mixed 2x PCR solution containing the recombinant heat stable Taq DNA polymerase from the thermophilic bacterium *Thermus aquaticus*, dNTPs, MgCl₂, and all other components required for PCR except primers and template DNA.

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

2. Applications

ROTI®Pol TaqHY Mix (2x) is perfectly suitable for all Taq-based cycling protocols with a) high yield, as being performed, for instance, prior to cloning, for amplicon elution, when using low-copy templates, or for educational purposes, or if b) particularly short cycle times are required.

PCR assays with ROTI®Pol TaqHY Mix (2x) master mix not only reduces contamination risks, but is also time-saving, highly reproducible and very easy to prepare.

Due to the optimised composition of the master mix, the TaqHY polymerase delivers specific PCR amplification of good yield with a wide range of PCR templates. ROTI®Pol TaqHY Mix (2x) is able to amplify PCR products up to 3 kb with genomic DNA, and is appropriate for use in the amplification of DNA from a wide range of even complex templates. The TaqHY polymerase included in the master mix 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.

The use of the colourless PCR master mix is particularly recommended when direct fluorescence or absorbance readings are required.

3. Content

ROTI®Pol TaqHY Mix (2x) (**Art. No. 1K33**) in **2x reaction buffer** containing TaqHY polymerase, 0,4 mM each dNTP, and 4 mM MgCl₂.

Filled in white-capped tubes.

Reagent	Lid colour	1K33.1	1K33.2
ROTI®Pol TaqHY Mix (2x)	white	2 tubes	10 tubes

The use of the colourless PCR master mix is adequate for all general PCR applications.

4. Reaction volume

The ready-to-use 2x master mix has been optimised for 25 µl reaction volumes. Use 12.5 µl of the 2x master mix solution and add up to 25 µl with primers, target DNA and water as described below.

5. Suggested pipetting scheme

Prepare on ice:

Components	Apply for PCR reaction of 25 µl volume	Final concentration (recommended)
ROTI®Pol TaqHY Master mix (2x)	12.5 µl	1x
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
Sterile dest. water	adjust to 25 µl final volume	

6. 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

7. 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. 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.

Further optimization may still be necessary by increasing MgCl₂ concentrations, primer concentrations and PCR cycle parameters depending on your DNA source and quality or your primers.

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.

8. Recommended MgCl₂ concentration

2-4 mM

When the 2x master mix is diluted 1:2 the final concentration of MgCl₂ is 2 mM. In case the MgCl₂ concentration has to be adjusted, use a separate MgCl₂ solution (100 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a MgCl₂ gradient in order to find the optimal concentration.

Pipetting scheme for additional MgCl₂

Final MgCl ₂ conc. in mM	2.5	3	3.5	4
Add 100 mM MgCl ₂ solution in following amounts to 25 µl reaction volume	0.125 µl	0.25 µl	0.375 µl	0.5 µl

9. Storage conditions

Store at -20 °C. Avoid extensive freeze/thaw cycles or prepare and store working aliquots. However, the master mix is stable for at least 8 freeze/thaw cycles.

Infrequent short term storage (few hours) of the master mix may be done at +4 °C.

10. 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 Master mixes:

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 TaqHY Red-Mix	Art. No. 1K34

ROTI®Pol TaqHY Mix (2x)	2 ml	2x Master mix	1K33.1
	10 ml	2x Master mix	1K33.2

Carl Roth GmbH + Co. KG

Schoemperlenstraße 3-5 • 76185 Karlsruhe • P.O. Box 100121 • 76231 Karlsruhe
Phone: +49 (0) 721/ 5606-0 • Fax: +49 (0) 721/ 5606-149 • info@carlroth.com • www.carlroth.com

The company is a limited partnership with headquarters in Karlsruhe, reg. court Mannheim HRA 100055. Roth Chemie GmbH, with headquarters in Karlsruhe, reg. court Mannheim HRB 100428, is the personally liable partner. Managing Director: André Houdelet. Sales tax identification number: DE 143621073.

jh 01/2022