Instructions for use

ROTI®Pol Hot-TaqS Mix (2x)

Recombinant hot start Taq DNA polymerase master mix, antibody blocked, for all PCR amplifications
9248

1. Description

Optimised pre-mixed 2x PCR solution containing the recombinant hot start Hot-TaqS DNA polymerase from the thermophilic bacterium *Thermus aquaticus*, dNTPs, $MgCl_2$, 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 TaqS Mix (2x) is recommended for use in all PCR applications, particularly if PCR is to be followed directly by gel electrophoresis.
PCR assays with ROTI®Pol Hot-TaqS Mix (2x) master mix not only reduces contamination risks, but is also time-saving, highly reproducible and very easy to prepare. ROTI®Pol Hot-TaqS Mix (2x) is superior for use in all standard Taq-based cycling protocols when big sample numbers shall be amplified with high specificity as well as high reproducibility. ROTI®Pol Hot-TaqS Mix (2x) master mix is, therefore, the optimal choice for high throughput PCR assays in colony screening, prior to sequencing, in cycle sequencing, and in similar assays.
Due to the optimised composition of the master mix combined with the antibody-blocked Hot-TaqS polymerase, ROTI®Pol Hot-TaqS Mix (2x) 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. Antibody concentration is adjusted for the effective inhibition of polymerase activity at temperatures up to 60°C. The polymerase is activated during normal cycling conditions, allowing for a convenient assembly of PCR reactions at room-temperature.
ROTI®Pol Hot-TaqS Mix (2x) is able to amplify PCR products up to 3 kb with genomic DNA and up to 5 kb with Lambda DNA, and is appropriate for use with pure DNA solutions, cDNA, and bacterial colonies as templates. The Hot-TaqS 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 Hot-TaqS Mix (2x) **(Art. No. 9248**) in **2x reaction buffer** containing hot start Hot-TaqS polymerase, 0,4 mM each dNTP, and 4 mM $MgCl_2$
Filled in white-capped tubes.

Reagent	Lid colour	9248.1	9248.2
ROTI®Pol Hot-TaqS 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

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 25 µl volume	Final concentration (recommended)
ROTI®Pol Hot-TaqS 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	

Gently vortex the sample and centrifuge briefly to collect all drops to the bottom of the tube. Place the samples in a thermocycler and start a PCR program.

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 $MgCl_2$ 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 $MgCl_2$ concentration

2-4 mM
When the 2x master mix is diluted 1:2 the final concentration of $MgCl_2$ is 2 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 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 cyles.
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 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 Mix (2x)	2 ml	2x Master mix	9248.1
	10 ml	2x Master mix	9248.2

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