ReverHotTaq DNA/ RNA dependent DNA Polymerase

Description: ReverHotTaq DNA Polymerase is an artificial engineered thermostable DNA polymerase, originated from BIORONs SD Polymerase. The HotStart function with AntiTaq antibody prevents non-specific amplification and allows reaction set-up at room temperature. ReverHotTaq shows a high reverse transcriptase activity for sensitive and robust cDNA synthesis and PCR amplification. Therefore, it can be used in a one-step RT-PCR assay with only one enzyme. The enzyme also possesses strand displacement activity and high thermostability up to 93 °C so it is suitable for RNA templates with a complex secondary structure. Both total RNA and mRNA can be used as template. ReverHotTaq allows to detect target transcripts in a single reaction with only 10 pg of total RNA using gene-specific primers.
It also provides efficient, robust and specific PCR and qPCR amplification of DNA templates. Due to the 5' - 3' exonuclease activity, ReverHotTaq can be used in qPCR or RT-qPCR with hydrolysis probes (e.g. TaqMan).

Concentration: 50x (15 U/µl)

Storage: -18 °C to - 22 °C. Avoid freeze-thaw cycles.

REF	106050	106055	colour
ReverHotTaq	**100 rcs**	**500 rcs**	**blue**
5x Rever Reaction Buffer	**500 µl**	**5x 500 µl**	**red**

Application: One-Tube RT-qPCR, RT-PCR, qPCR, PCR

We do not recommend to use ReverHotTaq in isothermal amplifications due to the 5'-3' exonuclease activity!

- For LAMP, PCDR or other isothermal amplifications we recommend our SD Polymerase REF 108702/ 108710 or SD Polymerase HotStart REF 108902/108910.
- Do not vortex the enzyme tube (blue) to avoid damaging the enzyme.

See next page for recommended standard protocol.

BIORON GmbH – In den Rauhweiden 20 - 67354 Römerberg (Germany)
Phone +49 6232 298 45 0 - Fax +49 6232 298 45 29
info@bioron.net www.bioron.net

Recommended Standard Protocol for RT-PCR/ PCR Mix:

Component	25 µl Reaction	Final Concentration
5x Reaction Buffer	5 µl	1 x
dNTP Mix (10 mM each)	0.6 µl	250 µM each
Gene-specific primers*	Variable	200 – 400 nM each
Probe (optional)	Variable	150 – 200 nM
Template (DNA or RNA)	Variable	0.02 – 250 ng per reaction (1 pg/µl - 10 ng/µl)**
50 x ReverHotTaq	0.5 µl	1 x
Nuclease-free ddH_2O	Adjust to 25 µl final volume	--

* Selecting primers with high melting temperature (60-68°C) is recommended
** Concentration for total RNA or gDNA

Recommended Thermocycler Protocol for PCR

Step	Time	Temperature	Cycles
Initial Denaturation	2 minutes	92 °C	1 x
Denaturation	30 seconds	92 °C	20 – 45 x
Annealing	30 seconds	58 – 68 °C*	
Extension	30 seconds per 1 kb amplicon length	68 - 70 °C	

* Depends on primer, the optimal annealing temperature is usually 2 – 5°C below the primer melting temperature

Recommended Thermocycler Protocol for RT-PCR

Step	Time	Temperature	Cycles
Initial Denaturation RT	2 minutes	75 °C*	1 x
Annealing RT	10 minutes	60 – 68 °C*	1 x
Extension RT	15 - 30 minutes	68 °C	1 x
Initial Denaturation PCR	1 minute	92 °C	1 x
Denaturation PCR	15 - 30 seconds	92 °C	30 - 50 x
Annealing/ Extension PCR	30 - 60 seconds	60 - 70 °C	

* Some complex RNA templates may benefit from initial 92 °C for 30 seconds

BIORON GmbH – In den Rauhweiden 20 - 67354 Römerberg (Germany)
Phone +49 6232 298 45 0 - Fax +49 6232 298 45 29
info@bioron.net www.bioron.net