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Medix Biochemica

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Product Manual
Cat. No: #6500

RevTaq RT-PCR DNA Polymerase

Description

RevTaq RT-PCR DNA polymerase is supplied as a 50x solution containing glycerol together with an optimized 5x reaction buffer. An aptamer-based hot-start formulation prevents non-specific amplification at ambient temperatures. Temperatures above 55°C cause the aptamer's secondary structure to melt and will set free the polymerase.

RevTaq RT-PCR DNA polymerase is an engineered, extremely thermostable reverse transcriptase and combined DNA polymerase, obtained through directed, artificial evolution. RevTaq RT-PCR DNA polymerase has a half-life at 95°C of >40 min.

RevTaq RT-PCR DNA polymerase allows “0-step” RT-PCRs directly from RNA templates (without an isothermal reverse transcription step), as reverse transcription takes place simultaneously with DNA amplification during the cycled PCR elongation step. This also facilitates reverse transcription reactions at high temperatures, thus minimizing the problems encountered with strong secondary structures in RNA that melt at elevated temperatures.

Kit components

Component	S pack	M pack
RevTaq DNA Polymerase	1 x 50 µL	1 x 250 µL
5x RevTaq RT-PCR DNA Polymerase Reaction Buffer	1 X 1.25 mL	2 X 1.25 mL

*Other pack sizes, bulk orders and customization are available upon request.

Storage and shipment

Transport with cool packs. The reagents should be stored at -20°C upon arrival. The reagents are stable until the expiration date if stored correctly.

Reaction master mix set-up

The recommended master mix set-up for a 25 µL reaction volume is shown in the table below.

Reagent	Volume (µL)	Final concentration
RevTaq DNA Polymerase (50x)	0.5	1x
5x RevTaq RT-PCR DNA Polymerase Reaction Buffer	5	1x
Forward primer (10 µM)	1.25	500 nM (50-1000 nM)
Reverse primer (10 µM)	1.25	500 nM (50-1000 nM)
Probe (10 µM)	x	50-1000 nM
dNTPs (10 nM)	0.625	250 µM
Template / Sample extract*	y	>0.1ng (0.1-2500 ng)
Nuclease-free water	Up to 25 µL final volume	

Keep all components on ice.

Spin down and mix all solutions carefully before use.

* Recommended template concentration should be 0.004 ng/µl – 0.1 µg/µl (of total RNA or genomic DNA).

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Instrument and program set-up

Cycles	Steps	Temperature	Time
RT Cycling			
10	Denaturation	95°C	3 sec
	Reverse Transcription*	65-80°C	60 sec
PCR Cycling			
35-50	Denaturation	95°C	10 sec
	Annealing / Extension **	58–75°C	50 sec

*This temperature is usually higher than the temperature during the PCR stage

**It is recommended to optimize the temperatures of the annealing/extension steps by applying a temperature gradient during establishments.

Technical information and support

Due to the aptamer-based hot-start formulation, RevTaq RT-PCR DNA polymerase will yield better results with annealing and extension temperatures above 57°C.

Since the enzyme is thermostable, it is recommended to design very high melting primers and probes (>65°C).

It is recommended to optimize the temperatures of the annealing/extension steps by applying a temperature gradient during establishments.

Higher temperatures lead to higher specificity of the PCR. The RT-cycles are usually run at higher temperatures than the PCR-cycles since DNA-primer: RNA-template hybrids generally have higher melting points than DNA-primer: cDNA template duplexes.

Depending on the employed assay, the reverse transcription steps may be omitted (0-step RT-PCR).

RevTaq RT-PCR DNA polymerase is engineered and optimized for an amplicon size between 60- 300 bp.