Medix Biochemica

Product Manual
Cat. No: #6601

HighScript Reverse Transcriptase

Description

HighScript Reverse Transcriptase together with enhanced buffer chemistry enables fast synthesis of a cDNA that accurately represents the transcript. The enzyme together with its buffer allows efficient and unbiased synthesis of the cDNA molecule. HighScript Reverse Transcriptase is a modified version of MMLV reverse transcriptase with noticeable thermostability and high enzymatic activity. This enzyme is offered as a blend with an RNase inhibitor to prevent RNA degradation. Total RNA is the preferred substrate of this enzyme because it is not inhibited by other forms of RNA (rRNA and/or tRNA).

Kit Components

Component	S pack*	M pack*
HighScript Reverse Transcriptase (200 U/µL) (with RNase inhibitor)	2 x 0.025 mL	2 x 0.1 mL
∞5x HighScript buffer	0.2 mL	4 x 0.2 mL

*Other pack sizes or bulk orders are available upon request.

∞The 5x HighScript buffer contains 15 mM MgCl2, 5 mM dNTPs, enhancers, and stabilizers. It was designed for robust performance; it is not recommended to add further enhancers.

Storage and Shipment

Transport with an ice pack. The reagents should be stored at -20°C upon arrival. The reagents are stable until the expiration date if stored correctly. Do not store the mix once it is combined with the RTase.

Reaction Master Mix Set-Up

The recommended master mix set-up for a 20 µL reaction volume is shown in the table below. To increase cDNA yield, the primer mix can be preincubated with the RNA template for 5 minutes at 70°C before cooling to 4°C. The reaction can be started by adding the reaction mix containing the other components to the preincubated primers and template. After combining the master mix with the template, the recommended reaction conditions for most applications (<65% GC) are 42°C for 30 minutes. For templates with more complex secondary structure, incubation up to 55°C may be used. Incubate at 85 °C for 10 minutes to denature the RTase.

Note for PCR set up: 4.0 µL of cDNA is recommended per 20 µL real-time PCR reaction or 50 µL endpoint PCR reaction.

Reagent	Volume (µL)	Final concentration
5x HighScript Buffer	4	1x
**HighScript Reverse Transcriptase (200 U/µL) (with RNase inhibitor)	1	200 U
4 pg to 0.4 µg of total RNA or oligodT-purified mRNA	x	Variable
∞∞ 10x Primer Mix	2	1x
Nuclease-free Water	Up to 20 µL final volume	

**It is suggested to add this component to the mix before total RNA as the RNase inhibitor is blended with RTase.

∞∞The suggested final primer concentration is 0.1 µM for specific primers, 1 µM for Olido-dT18, and 2–5 µM for random hexamers.

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Technical information and support

For technical enquiries or assay development support, please contact us via e-mail at: mdx@medixbiochemica.com.

Additional information and technical resources are available on our website at: info.medixbiochemica.com/resources.

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