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UltraScript Reverse Transcriptase

Product description:

UltraScript Reverse Transcriptase uses the latest developments in reverse transcriptase technology and buffer chemistry to enhance cDNA synthesis speed and yield with accurate transcript representation. The reverse transcriptase buffer system allows for efficient, non-biased and sensitive cDNA synthesis.

UltraScript Reverse Transcriptase is a modified MMLV reverse transcriptase (RTase) that is both thermostable and extremely active. The RTase is not inhibited by ribosomal and transfer RNAs, making total RNA an ideal substrate. The enzyme is blended with RNase inhibitor preventing degradation of RNA by contaminating RNase.

The 5x buffer contains enhancers, dNTPs and $MgCl_2$. It does not contain oligos. The kit can be used with 4.0 pg to 0.4 µg total RNA or oligo(dT) purified mRNA. However, the optimal tempate concentration will ultimately be determined by what oligos are used.

Component	10 000 units	40 000 units
5x UltraScript Buffer	1 x 200 µL	4 x 200 µL
UltraScript (200units/µL) (with RNAse inhibitor)	2 x 25 µL	2 x 100 µL

Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. If stored correctly, the kit will retain full activity until the indicated expiry date. Avoid exposure of the stock solution to frequent temperature changes and limit handling at room temperature to the necessary minimum.

Limitations of product use

The product may be used for in vitro research purposes only.

Technical support

Help is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting please email technical@pcrbio.com with the following information:

- Reaction setup
- PCR cycling conditions
- Screen grabs of gel images / real-time PCR traces

Important considerations

5x UltraScript Buffer: Contains 15 mM $MgCl_2$, 5 mM dNTPs, enhancers and stabilizers. It is not recommended to add further enhancers or $MgCl_2$ to the reaction. The buffer composition has been optimised to generate high yield, non-biased cDNA for downstream applications.

Primers: Suggested primer concentrations are in the table below. For non-biased, non-specific amplification, we recommend using both random hexamers and oligo-dT_{18}.

Oligo Type	Reaction Concentration	10x Stock Concentration
Specific Primers	0.1 µM	1 µM
Random Hexamers	2 - 5 µM	20 - 50 µM
Oligo-dT_{18}	1 µM	10 µM

Template: Use 4.0 pg to 0.4 µg total RNA or oligo(dT) purified mRNA. For template amounts greater than 0.4 µg we recommend UltraScript 2.0 Reverse Transcriptase.

Optional preincubation: Incubating primer mix with template for 5 minutes at 70 ˚C before adding to reaction mix will increase cDNA yield. However, this step is not necessary for accurate quantification.

Incubation temperature: We recommend incubating with a temperature of 42 ˚C for 30 minutes for the majority of applications (<65% GC). Where regions of interest contain high secondary structure (>65% GC) incubation temperatures of up to 55 ˚C may be used.

PCR setup: We recommend 4.0 µL of cDNA per 20 µL real-time PCR reaction and 50 µL endpoint PCR reaction

Reaction Setup

1. Allow 5x UltraScript Buffer to thaw, briefly vortex.
2. Prepare a master mix based on the following table. Insert reagents in sequence listed:

Reagent	20 µL reaction	Final concentration	Notes
5x UltraScript Buffer	4.0 µL	1x	
UltraScript (200units/µL) (with RNAse inhibitor)	1.0 µL		Add before total RNA as RNase inhibitor is blended with RTase
4.0pg to 0.4µg Total RNA or oligo(dT) purified mRNA	X µL		
10x Primer Mix	2 µL	1x	See Primers section
PCR grade dH_2O	Up to 20 µL final volume		

No RT control setup (optional)

Reagent	20 µL reaction	Final concentration	Notes
5x UltraScript Buffer	4.0 µL	1x	
4.0 pg to 0.4 µg Total RNA or oligo(dT) purified mRNA	X µL		Use equal amount as in step 2
10x Primer Mix	2 µL	1x	Use equal amount as in step 2
PCR grade dH_2O	Up to 20 µL final volume		

Incubation and enzyme denaturation

3. Incubate at 42 ˚C for 30 minutes.
4. Incubate at 85 ˚C for 10 minutes to denature RTase.