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UltraScript Reverse Transcriptase

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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 4.0pg to 0.4 μ g total RNA or oligo(dT) purified mRNA. However, the optimal template concentration will ultimately be determined by what oligos are used.

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

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 for 12 months. 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 15mM MgCl₂, 5mM dNTPs, enhancers and stabilizers. It is not recommended to add further enhancers or MgCl₂ 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-dT₁₈.

Oligo Type	Reaction Concentration	10x Stock Concentration
Specific Primers	1pM	10pM
Random Hexamers	2 - 5μM	20 - 50μM
Oligo-dT ₁₈	1μM	10μM

Template: Use 4.0pg to 0.4μg total RNA or oligo(dT) purified mRNA. For template amounts greater than 0.4μg 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 4.0μL of cDNA per 20μL real-time PCR reaction and 50μL 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	20μL reaction	Final concentration	Notes
5x UltraScript Buffer	4.0μL	1x	
UltraScript (200units/μL) (with RNase inhibitor)	1.0μL		Add before total RNA as RNase inhibitor is blended with RTase
4.0pg to 0.4μg Total RNA or oligo(dT) purified mRNA	XμL		
10x Primer Mix	2μL	1x	See Primers section
PCR grade dH ₂ O	Up to 20μL final volume		

No RT control setup (optional)

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