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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.

Quality control

PCR Biosystems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

Pack size	5x UltraScript® Buffer	UltraScript® (200 U/µL) (with RNase inhibitor)
10000 units	1 x 200 µL	2 x 25 µL
40000 units	4 x 200 µL	2 x 100 µL

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °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. Do not store the mix once it is combined with the RTase.

Technical support

Scan or click the QR codes for helpful cDNA synthesis tips and answers to frequently asked technical questions. For further technical support, please email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile

TIPS & TRICKS

FAQS

Important considerations

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

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

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 (templates with <65% GC). Incubation temperatures of up to 55 °C may be used only when the regions of interest contain high secondary structure (>65% GC), as this can increase cDNA yields. Using high temperatures for templates with <65% GC might reduce the yield of transcription.

PCR setup: Add up to 4 µL of cDNA per 20 µL qPCR reaction or 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 µL	1x	
UltraScript® (200 U/µL) (with RNAse inhibitor)	1 µL		Add before total RNA as RNase inhibitor is blended with RTase
4 pg to 0.4 µg 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 (recommended for qPCR)

3. 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 µL	1x	
4 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

4. Incubate at 42 °C for 15 - 30 minutes. Longer incubation times increase cDNA yield.
5. Incubate at 85 °C for 10 minutes to denature RTase