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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 4.0pg to 0.4µg 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 200µl	4 x 200µl
UltraScript (200units/µl) (with RNAse inhibitor)	2 x 25µl	2 x 100µl

Shipping and storage

On arrival the kit should be stored between -30˚C and -15˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4˚C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

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

Technical support

For technical support and troubleshooting please email technical@pcrbio.com the following information:

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

Important considerations

5x UltraScript Buffer: Contains 15mM $MgCl_2$, 5mM 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	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_2O	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_2O	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.