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UltraScript 2.0 cDNA Synthesis Kit

Product description:

UltraScript 2.0 cDNA Synthesis Kit is designed for fast, reliable and unbiased cDNA synthesis from a wide range of RNA sample types. The kit contains all the required components for cDNA synthesis, including a combination of anchored oligo(dT)$_{18}$ and random hexamers optimised to produce high quality cDNA for use in real-time PCR applications.

The kit utilizes UltraScript 2.0 Reverse Transcriptase (RTase), a robust and highly thermostable modified MMLV reverse transcriptase engineered for superior cDNA synthesis speed, yield and representation. The RTase is blended with an advanced RNase inhibitor to prevent degradation of RNA by contaminating RNase.

UltraScript 2.0 RTase is not inhibited by ribosomal and transfer RNAs making total RNA an ideal substrate. The kit can be used with 20pg to 3.5µg total RNA or oligo(dT) purified mRNA.

The 5x buffer contains enhancers, anchored oligo(dT)$_{18}$, random hexamers, dNTPs and $MgCl_2$. The relative concentrations of random hexamers and anchored oligo(dT)$_{18}$ have been optimised for unbiased cDNA synthesis for use in subsequent real-time PCR experiments.

Component	25 reactions	100 reactions
5x cDNA Synthesis Mix	1 x 100µl	4 x 100µl
UltraScript 2.0 for cDNA Synthesis (with RNase inhibitor)	1 x 25µl	1 x 100µl

Shipping and storage

On arrival the kit should be stored at -20˚C. Avoid prolonged exposure to light. 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 cDNA Synthesis Mix: Contains anchored oligo(dT)$_{18}$, random hexamers, 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.

Template: Use 20pg to 3.5µg total RNA or oligo(dT) purified mRNA for accurate quantification. Additional RNA is not recommended for quantification, as total reverse transcription is not guranteed. As concentrations of target sequences will vary, users are encouraged to perform a template titration to find the optimal concentration for their application.

Optional preincubation: Incubating template with primers prior to reverse transcription can increase the amount of cDNA, however this step is not necessary for accurate quantification. If preincubation is desired, incubate template with 5x cDNA Synthesis Mix for 2 minutes at 70˚C, then rapidly cool to 4˚C, before adding the reverse transcriptase.

Incubation temperature: We recommend incubating with a temperature of 50˚C for 30 minutes for most applications. Where regions of interest contain high secondary structure (>65% GC), incubation temperatures of up to 70˚C may be used, but this will reduce the activity of the enzyme and may result in less total cDNA. The same temperature should be used when comparing samples.

PCR setup: We recommend adding 2.0-4.0µl of cDNA solution to a 20µl qPCR reaction. As excess RTase can inhibit Taq activity, better sensitivity can sometimes be obtained by diluting the resulting cDNA. We recommend diluting the cDNA 10x-100x when quantifying genes with low expression.

Reaction Setup

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

Reagent	20µl reaction	Final concentration	Notes
5x cDNA Synthesis Mix	4.0µl	1x	
UltraScript 2.0 for cDNA Synthesis (with RNase inhibitor)	1.0µl		Add before total RNA as RNase inhibitor is blended with RTase
20pg to 3.5µg Total RNA or oligo(dT) purified mRNA	Xµl		
PCR grade dH_2O	Up to 20µl final volume		

No RT control setup (optional)

Reagent	20µl reaction	Final concentration	Notes
5x cDNA Synthesis Mix	4.0µl	1x	
20pg to 3.5µg Total RNA or oligo(dT) purified mRNA	Xµl		Use equal amount as in step 2
PCR grade dH_2O	Up to 20µl final volume		

Incubation and enzyme denaturation

3. Incubate at 50-55˚C for 10-30 minutes.
4. Incubate at 95˚C for 10 minutes to denature RTase.