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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 utilises 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 100 µL	4 x 100 µL
UltraScript 2.0 for cDNA Synthesis (with RNase inhibitor)	1 x 25 µL	1 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. Do not store the mix once it is combined with the RTase.

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 cDNA Synthesis Mix: Contains anchored oligo(dT)$_{18}$, random hexamers, 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.

Template: Use 20 pg to 3.5 µg 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: UltraScript 2.0 contains a high concentration of RTase. Because excess RTase can inhibit DNA polymerase activity, we strongly recommend diluting the cDNA 10x-100x when it is to be used in PCR or qPCR reactions, even for low copy number gene expression analysis.

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

No RT control setup (optional)

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