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UltraScript® cDNA Synthesis Kit

Product description

The UltraScript® cDNA Synthesis Kit enables enhanced cDNA synthesis speed and yield with accurate transcript representation. The reverse transcriptase, buffer system and combination of random hexamers with anchored oligo(dT) allow for unbiased, efficient and sensitive cDNA synthesis.

The modified MMLV reverse transcriptase (RTase) 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 supplied 5x buffer contains anchored oligo(dT), random hexamers, enhancers, dNTPs and $MgCl_2$. The relative concentrations of random hexamers and anchored oligo(dT) have been optimised for the generation of cDNA for use in real-time PCR experiments. The kit can be used with 4.0 pg to 0.4 µg total RNA or oligo(dT) purified mRNA. Generated cDNA can be used for qPCR, as a template for cloning or for library construction. This kit can be used with both eukaryotic and prokaryotic RNA.

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 cDNA Synthesis Mix	20x UltraScript® for cDNA Synthesis
25 reactions	1 x 100 µL	1 x 25 µL
100 reactions	1 x 400 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 cDNA Synthesis Mix: Contains anchored oligo(dT), 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 cDNA for downstream real-time PCR analysis.

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 our UltraScript 2.0 cDNA Synthesis Kits. Alternatively, reactions can be scaled up to a larger volume to accomodate greater RNA input, with a proportional increase of all reagent volumes, as recommended it example reaction setup below.

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.

qPCR setup: Users can add the generated cDNA directly to a qPCR reaction, or dilute it 10x - 50x in PCR grade H_2O to reduce the concentration and extend the volume. We recommend adding 2 - 4 µL of cDNA solution to a 20 µL qPCR reaction.

Reaction setup

1. Allow 5x cDNA Synthesis Mix 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 cDNA Synthesis Mix	4 µL	1x	
20x UltraScript® for cDNA Synthesis	1 µL		Add before total RNA as RNase inhibitor is blended with RTase
Total RNA or oligo(dT) purified mRNA (between 4.0 pg and 0.4 µg)	X µL		
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 cDNA Synthesis Mix	4 µL	1x	
Total RNA or oligo(dT) purified mRNA (between 4.0 pg and 0.4 µg)	X µL		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