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UltraScript™ cDNA Synthesis Kit Separate Oligos

Product description:

The UltraScript™ cDNA Synthesis Kit Separate Oligos uses the latest developments in reverse transcriptase technology and buffer chemistry to enhance cDNA synthesis speed and yield with accurate transcript representation. The kit contains all the required components for cDNA synthesis, including separate anchored oligo(dT)$_{18}$ and random hexamers for unbiased, efficient and sensitive cDNA synthesis for many different downstream applications.

The kit utilises UltraScript™ Reverse Transcriptase (RTase), a modified MMLV reverse transcriptase which 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 $MgCl_2$. The anchored oligo(dT)$_{18}$ and random hexamers are provided separately. The kit can be used with the oligos provided, or with primers of the user's design. The kit can be used with 4.0 pg to 0.4 µg total RNA or oligo(dT) purified mRNA.

Component	25 reactions	100 reactions
5x UltraScript™ Buffer	1 x 200 µL	2 x 200 µL
20x UltraScript™ for cDNA Synthesis	1 x 25 µL	1 x 100 µL
100 µM Anchored Oligo(dT)$_{18}$	1 x 100 µL	1 x 100 µL
100 µM Random Hexamers	1 x 100 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 and support is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting you can submit a technical enquiry online, or alternatively 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 UltraScript™ Buffer: Contains 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 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	0.1 µM	1 µM
Random Hexamers	1-5 µM	10-50 µM
Oligo$(dT)_{18}$	50-500 nM	0.5-5 µM

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.

qPCR setup: Users can add the cDNA created 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 2.0 - 4.0 µL of cDNA solution to a 20 µL qPCR 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	4.0 µL	1x	
20x UltraScript™ for cDNA Synthesis	1.0 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		
10x Primer Mix	2 µL	1x	See Primers section above
PCR grade dH_2O	Up to 20 µL final volume		

No RT control setup (optional)

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	4.0 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 30 minutes
5. Incubate at 85 ˚C for 10 minutes to denature RTase