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UltraScript 2.0 cDNA Synthesis Kit Separate Oligos

Product description:

UltraScript 2.0 cDNA Synthesis Kit Separate Oligos is designed for fast and reliable cDNA synthesis from a wide range of RNA sample types. The kit contains all the required components for cDNA synthesis, including separate anchored oligo(dT)$_{18}$ and random hexamers, to produce high quality cDNA suitable for many different downstream 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, 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.

Component	25 reactions	100 reactions
5x UltraScript Buffer	1 x 200 µL	2 x 200 µL
UltraScript 2.0 for cDNA Synthesis (with RNase inhibitor)	1 x 25 µL	2 x 100 µL
100µM Anchored Oligo(dT)$_{18}$	1 x 100 µL	1 x 100 µL
100µM 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 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 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

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 primers for 2 minutes at 70 ˚C, then rapidly cool to 4 ˚C, before adding to reaction.

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 4.0 µL of cDNA per 20 µL PCR 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 UltraScript Buffer 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 UltraScript Buffer	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		
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)

Reagent	20 µL reaction	Final concentration	Notes
5x UltraScript Buffer	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
10x Primer Mix	2 µL	1x	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.