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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 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, 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 200µl	2 x 200µl
UltraScript 2.0 for cDNA Synthesis (with RNase inhibitor)	2 x 25µl	2 x 100µl
100µM Anchored Oligo(dT)$_{18}$	1 x 100µl	1 x 100µl
100µM Random Hexamers	1 x 100µl	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 UltraScript Buffer: Contains 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 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	1pM	10pM
Random Hexamers	1-5µM	10-50µM
Oligo$(dT)_{18}$	50-500nM	0.5-5µM

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 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 4.0µl of cDNA per 20µl 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	20µl reaction	Final concentration	Notes
5x UltraScript Buffer	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		
10x Primer Mix	2µl	1x	See Primers section above
PCR grade dH_2O	Up to 20µl final volume		

No RT control setup (optional)

Reagent	20µl reaction	Final concentration	Notes
5x UltraScript Buffer	4.0µl	1x	
20pg to 3.5µg Total RNA or oligo(dT) purified mRNA	Xµl		Use equal amount as in step 2
10x Primer Mix	2µl	1x	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.