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UltraScript 2.0 Reverse Transcriptase

Product description:

UltraScript 2.0 Reverse Transcriptase (RTase) is a robust and highly thermostable modified MMLV reverse transcriptase engineered for superior cDNA synthesis speed, yield and representation from a wide range of RNA sample types.

UltraScript 2.0 Reverse Transcriptase is provided with an advanced 5x buffer containing enhancers, dNTPs and $MgCl_2$ designed to give sensitive and efficient cDNA synthesis from a broad range of RNA input amounts. As oligos are not included, users have the flexibility to define their own priming strategy.

UltraScript 2.0 Reverse Transcriptase is not inhibited by ribosomal and transfer RNAs, making total RNA an ideal substrate. The RTase can be used with 20 pg to 3.5 µg total RNA or oligo(dT) purified mRNA, however, the optimal tempate concentration will ultimately be determined by what oligos are used.

The RTase is blended with an advanced RNase inhibitor preventing degradation of RNA by contaminating RNase.

Component	10 000 units	40 000 units
5x UltraScript Buffer	1 x 200 µL	4 x 200 µL
UltraScript 2.0 (200units/µL) with RNase inhibitor	2 x 25 µL	2 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 only for in vitro research purposes.

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 $MgCl_2$, 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	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: 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 UltraScript Buffer to thaw, 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 (200 units/µL) 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
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.