PCRBIOSYSTEMS
simplifying research

qPCRBIO cDNA Synthesis Kit

Product description:

The qPCRBIO cDNA Synthesis Kit uses the latest developments in reverse transcriptase technology and buffer chemistry to enhance 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.

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 4.0pg to 0.4µg total RNA or oligo(dT) purified mRNA.

Component	25 reactions	100 reactions
5x cDNA Synthesis Mix	100µl	400µl
20x RTase (with RNase inhibitor)	25µl	100µl

Shipping and storage

On arrival the kit should be stored at -20˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4˚C for 1 month. 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 cDNA Synthesis Mix: Contains anchored oligo(dT), random hexamers, 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 cDNA for downstream real-time PCR analysis.

Template: Use 4.0pg to 0.4µg total RNA or oligo(dT) purified mRNA. Up to 5µg of total RNA may be added for increased cDNA yield, however complete reverse transcription of these high amounts is not guaranteed.

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 - 4.0µl of cDNA solution to a 20µl 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	20µl reaction	Final concentration	Notes
5x cDNA Synthesis Mix	4.0µl	1x	
20x RTase	1.0µl		Add before total RNA as RNase inhibitor is blended with RTase
Total RNA or oligo(dT) purified mRNA (between 4.0pg and 0.4µg)	Xµl		
PCR grade dH_2O	Up to 20µl final volume		

No RT control setup (optional)

3. Prepare a master mix based on the following table. Insert reagents in sequence listed:

Reagent	20µl reaction	Final concentration	Notes
5x cDNA Synthesis Mix	4.0µl	1x	
Total RNA or oligo(dT) purified mRNA (between 4.0pg and 0.4µg)	Xµl		Use equal amount as in step 2
PCR grade dH_2O	Up to 20µl 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