qPCRBIO **cDNA Synthesis Kit**

- Unbiased cDNA synthesis for real-time PCR
- Thermostable reverse transcriptase
- 30 minute protocol

Features

- Unbiased representation of 5' and 3' mRNA transcript ends
- Sensitive detection of low copy number transcripts
- High cDNA yields from as little as 4pg total RNA
- Simple 2 tube system
- 5x buffer contains anchored oligo(dT), random hexamers, enhancers, dNTPs and $MgCl_2$
- 20x thermostable reverse transcriptase blended with RNase inhibitor

Applications

- cDNA synthesis for real-time PCR analysis
- Low copy number transcripts
- Viral RNA targets
- miRNA targets
- Efficient synthesis from total RNA or poly(A)+ RNA

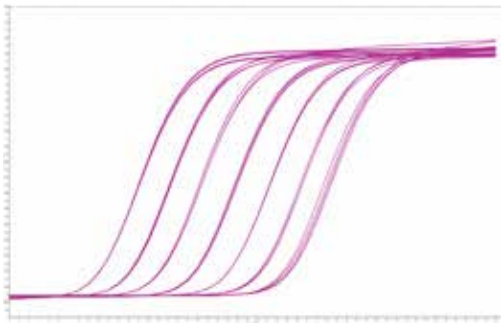


Figure 1. Broad reverse transcription dynamic range

A 10 fold serial dilution of mouse total RNA was used for 7 cDNA synthesis reactions. The highest amount of RNA used for cDNA synthesis was 4µg and the lowest 4pg.

Each cDNA synthesis reaction was used as a template for a qPCRBIO SyGreen reation, amplifying a 70bp fragment of the mouse RN18S gene. Reactions were incubated for 30minutes at 42˚C. Efficiency was measured at 100% across the range tested.

The results demonstrate that qPCRBIO cDNA Synthesis Kit efficiently reverse transcribes RNA across a broad dynamic range of substrate.

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The qPCRBIO cDNA Synthesis Kit uses the latest developments in reverse transcriptase technology and buffer chemistry to enhance cDNA synthesis speed, yield and representation. The reverse transcriptase, buffer system and combination of random hexamers with anchored oligo(dT) allow for unbiased, efficient and sensitive cDNA synthesis.

High quality cDNA synthesis for downstream qPCR analysis is essential for successful expression studies. Many factors affect cDNA synthesis including the reverse transcripase, buffer systems, enhancers and priming strategy. The PCRBIO cDNA synthesis mix removes the need for user optimisation of these critical factors.

The modified MMLV reverse transcriptase (RTase) is both thermostable and extremely active. The enzyme is blended with RNase inhibitor preventing degradation of RNA by contaminating RNase. The RTase is not inhibited by ribosomal and transfer RNAs, total RNA is an ideal substrate. The 5x cDNA synthesis mix can be used with up to 4µg total RNA.

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.

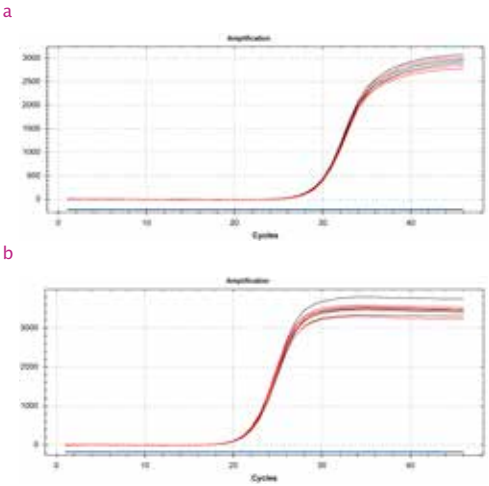


Figure 2. Unbiased representation of mRNA ends

a) qPCRBIO cDNA Synthesis Kit was used to synthesise cDNA from mouse liver total RNA. 2 primer pairs were designed against the 5' (red traces) and the 3' (black traces) ends of the 4.2kb mouse CANX transcript. qPCRBIO SyGreen Mix was used for analysis. The primer pairs were 4kb apart and did not show any reverse transcription bias, hence the amplification traces overlap.

b) 2 primer pairs against the 5' (red) and 3' (black) traces of RNS18 gene (1.8kb). Again, no reverse transcription bias was evident.

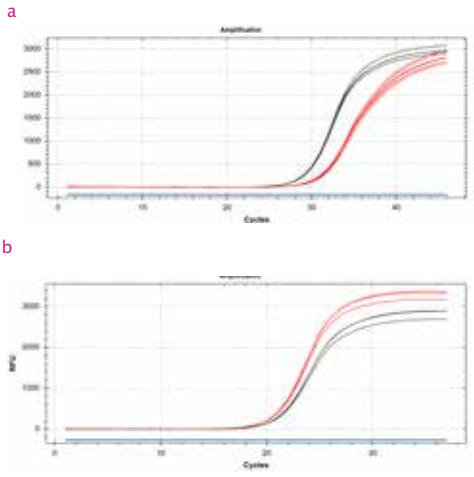


Figure 3. Thermostable enzyme for high GC%

a) qPCRBIO cDNA synthesis kit was used to synthesise cDNA from mouse liver total RNA at 42˚C (red) and also at 55˚C (black). A primer pair was designed against GJB2, generating an 84% GC amplicon. qPCRBIO SyGreen Mix was used for analysis. The higher temperature incubation generated more GC rich cDNA than the low temperature incubation.

b) A control amplicon of 55% GC from GAPDH was amplified from the 2 cDNAs described above. For this GC% no advantage of higher temperature incubation was achieved. The yield was slightly lower with the higher temperature.

Catalogue Number	Product Name	Pack size	Presentation
PB30.11-02	qPCRBIO cDNA Synthesis Kit	25 Reactions	[1 x 0.1ml] & [1 x 0.025ml]
PB30.11-10		100 Reactions	[4 x 0.1ml] & [1 x 0.1ml]

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