appleton®

For the Life Scientist

AppScript cDNA synthesis kit

High yield unbiased cDNA synthesis

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High Yield Unbiased cDNA for Your Downstream qPCR

AppScript cDNA synthesis kit contains a specially engineered MMLV reverse transcriptase (RTase) with both increased thermal stability and enhanced cDNA synthesis efficiency. The RTase is not significantly inhibited by ribosomal and transfer RNA and so can be used to synthesize cDNA from total RNA. The RTase contains a ribonuclease inhibitor to safeguard against the degradation of target RNA due to ribonuclease contamination. The kit comes with a 5x App cDNA mix containing pre-optimised oligo dT and random primers, $MgCl_2$, dNTPs, enhancers and stabilisers for the generation of cDNA for downstream qPCR.

www.appletonwoods.co.uk/qPCRselectionguide.png

Main Features

- For reproducible and unbiased cDNA synthesis from 5' and 3' ends of mRNAs
- Contains an ultra-stable reverse transcriptase which can synthesize cDNAs up to 9kb
- High cDNA yields from as little as 5pg RNA - easily work with low copy templates
- Broad starting template range – use between 5pg – 5μg
- The kit contains a buffer which has pre-optimised levels of oligo dT and random hexamers which results in reduced transcript bias for downstream qPCR
- Convenient two tube format:
  - o Tube 1: reverse transcriptase with RNase inhibitor to prevent degradation of RNA by contaminating RNases
  - o Tube 2: buffer concentrate containing oligo dT, random hexamers,$MgCl_2$, dNTPs, enhancers and stabilizers
- Short protocol (30mins only)

Ordering Information

Component	ARP601	ARP602	ARP602
20x App RTase (with RNAse inhibitor)	1x 25μL	4x 25μL	10x 25μL
5x App cDNA mix	1x 100μL	4x 100μL	10x 100μL
Pack Size	25 reactions	100 reactions	250 reactions

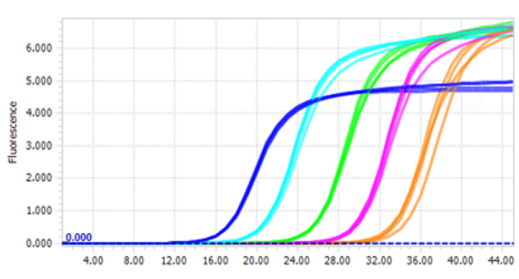

Figure 1a

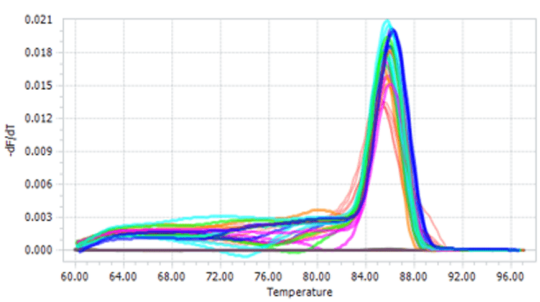

Figure 1b

Figure 1a & 1b : qPCR amplification curves (1a) and corresponding melt curves (1b) of serial dilutions of mouse cDNA made using the AppScript cDNA synthesis kit.
A 10 fold serial dilution of mouse total RNA was used for 4 cDNA synthesis reactions. The highest amount of RNA used for cDNA synthesis was 5μg and the lowest 5pg. Each cDNA synthesis reaction was used as a template for an AppGreen reaction, amplifying an 80bp fragment of the mouse GAPDH gene. Reactions were incubated for 30 minutes at 42˚C. The amplification curves are four 10 fold serial dilutions of cDNA prepared from total mouse RNA. The equal spacing in Figure 1a demonstrates close to 100% amplification efficiency. The overlapping melt curves demonstrate that the same product was produced from each template dilution. AppScript cDNA synthesis kit efficiently produces cDNA from a wide range of RNA concentrations.

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