



For the Life Scientist

## AppScript cDNA synthesis kit

### ORDERING INFORMATION

Component	ARP601	ARP602	ARP602
20x App RTase (with RNase inhibitor)	1x 25 $\mu$ L	4x 25 $\mu$ L	10x 25 $\mu$ L
5x App cDNA mix	1x 100 $\mu$ L	4x 100 $\mu$ L	10x 100 $\mu$ L
Pack Size	25 reactions	100 reactions	250 reactions

**Store at -20°C.** (The kit will retain full activity for 12 months at -20°C. Can be stored at 4°C for 1 month and go through 30 freeze/thaw cycles with no loss of activity. Avoid prolonged exposure to light).

### DESCRIPTION

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<sub>2</sub>, dNTPs, enhancers and stabilisers for the generation of cDNA for downstream qPCR.

### PROTOCOL

Prepare a master mix by mixing 5x App cDNA mix, 20x App RTase, template RNA and molecular biology grade water. To verify the results of the cDNA synthesis steps, set up a no-template control (NTC) containing only every reagent for the reverse transcription to assess for reagent contamination. To assess for genomic DNA contamination of the RNA sample set up a no RTase (NRT) control which contains every reagent for the reverse transcription (RT) except for the RTase.

1. Gently vortex and briefly centrifuge all solutions after thawing. Store on ice.
2. Place a sterile, nuclease-free microcentrifuge tube on ice and add the following components sequentially for each 20 $\mu$ L reaction.

#### Important:

1. Sequential addition of the RTase before the template RNA is necessary because the RTase is blended with an RNase inhibitor.
2. Template Amounts\*: Use 4.0pg - 0.1 $\mu$ g for total RNA or 0.2pg - 2.0 $\mu$ g for oligo (dT) purified mRNA.



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### cDNA synthesis using template RNA

Reagent	Final Concentration	20µL reaction
5x App cDNA mix	1X	4.0µL
20x App RTase (with RNase inhibitor)	1X	1.0µL
Template RNA	0.2pg – 2.0µg*	Variable (see above notes)
Molecular Biology Grade water, (BMW001)		Up to 20µl final volume

### No-template control (NTC)

Reagent	Final Concentration	20µL reaction
5x App cDNA mix	1X	4.0µL
20x App RTase (with RNase inhibitor)	1X	1.0µL
Molecular Biology Grade water, (BMW001)		Up to 20µl final volume

### No RTase (NRT) control

Reagent	Final Concentration	20µL reaction
5x App cDNA mix	1X	4.0µL
Template RNA	0.2pg – 2.0µg*	variable
Molecular Biology Grade water, (BMW002)		Up to 20µl final volume

3. Gently mix the samples and spin down.

4. Incubate the samples at 42°C for 30 mins. This is suitable for templates which are less than 65% GC-rich. For GC-rich RNA templates the reaction temperature can be increased up to 55°C.

5. Inactivate the RTase by heating at 85°C for 10 mins. The cDNA product can be directly used in subsequent PCR/ qPCR applications or stored at -20°C for less than one week, or at -70°C longer term. Use 4.0µl of cDNA per 20µl real-time PCR / qPCR reaction.

### CONSIDERATIONS

RNA can be degraded by RNase A, which is a highly stable contaminant found in most labs. All kit components have been tested to ensure that they are RNase free.

### General recommendations

- DEPC-treat all tubes and pipette tips to be used in cDNA synthesis or use certified nuclease-free consumables.
- Wear gloves when handling RNA and all reagents, as skin is a common source of RNases. Change gloves frequently.
- Use RNase-free reagents, including high quality water (e.g. nuclease-free (BMW002)).
- Keep all kit components tightly sealed when not in use. Keep all tubes tightly closed during the reverse transcription reaction.

### TROUBLE SHOOTING / TECHNICAL SUPPORT

For troubleshooting please visit [www.appletonwoods.co.uk/RTtroubleshooting.pdf](http://www.appletonwoods.co.uk/RTtroubleshooting.pdf) for a trouble shooting guide on RT. If this does not resolve your issues, please email [technicalsupport@appletonwoods.co.uk](mailto:technicalsupport@appletonwoods.co.uk) with details of your reaction setup, reaction conditions, gel images.

### ASSOCIATED PRODUCTS

Product	Pack Size	Product Code
Molecular Biology Grade Agarose	500g	AG001
Hot AppTaq Polymerase	250 units	ARP021
AppGreen Low ROX Mix (2X)	200 reactions	ARP102
Molecular biology grade water	500mL	BMW002

More pack sizes available at [www.appletonwoods.co.uk](http://www.appletonwoods.co.uk)