appleton®
For the Life Scientist

appGREEN 1-Step Extreme Low ROX Kit

ORDERING INFORMATION

Component	ARP742	ARP743
appGREEN 1-Step Low ROX Mix (2x)	200 reactions (2 x 1ml)	500 reactions (5 x 1ml)
20x RTase Extreme (contains RNase inhibitor)	2 x 200µl	5 x 200µl

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

appGREEN 1-Step Extreme Low ROX Kit is a high performance RT-qPCR reagent which has been optimised to be fast, specific and sensitive. It contains modified MMLV reverse transcriptase which is highly thermostable and extremely active at the same time.

The enzyme is blended with RNase inhibitor, which prevents degradation of RNA by contaminating RNase. The RTase is not inhibited by ribosomal and transfer RNAs, total RNA is an ideal substrate. All appGREEN 1-Step Extreme Kits contain an antibody-mediated hot start polymerase, which has been precisely engineered for highly specific qPCR and works in fast or standard thermal cycling conditions.

For the Life Scientist
appleton®

It also contains a proprietary intercalating dye, which does not interfere with or inhibit qPCR. It has been validated on various qPCR instruments - for a full list visit our website.

PROTOCOL

Prepare a RT-qPCR master mix by mixing molecular biology grade water, appGREEN 1-Step Low ROX Mix (2X), and forward and reverse primers. Prepare sufficient master mix for the experimental sample number of reactions, RNA standard reactions and no-template and no-RTase negative controls. Aliquot the master mix into individual PCR tubes / wells and then add template RNA/ molecular biology grade water for negative controls.

1. Gently mix and briefly centrifuge all solutions after thawing.

2. Add the following components for each 20µL reaction to a thin-walled, optically clear PCR tube/plate:

Reagent	Final Concentration	20µL reaction
appGREEN 1 Step Low ROX Mix (2X)	1X	10.0µL
Forward primer (10µM)	400nM	0.8µL
Reverse primer (10µM)	400nM	0.8µL
20x RTase Extreme (contains RNase inhibitor)	1X	1.0µL
Template RNA	1pg to 10ng total RNA >0.01pg mRNA	variable*
Molecular Biology Grade water, (BMW001)		Up to 20µl final volume

3. Gently mix the samples and spin down. Do not vortex as bubbles will interfere with fluorescence detection.

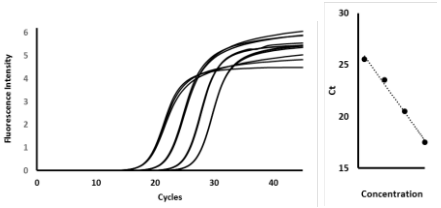
4. Perform RT-qPCR using recommended thermal cycling conditions as follows

Step	Temperature/ °C	Time	Cycles
Reverse transcription	45-55	10 min	1
Initial denaturation and enzyme activation	95	2 min	1
Denaturation	95	5 s	40
Annealing/ Extension	60-65	20-30 s	

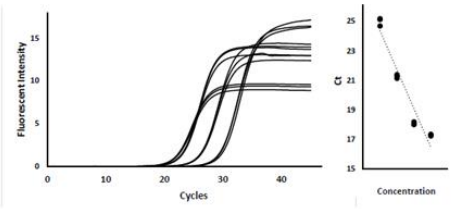
CONSIDERATIONS

Template*

Select the correct template concentration to correctly quantify the target sequence as the target copy number will be variable. An optimum template concentration will display clear separation between amplification curves (Fig.1). At lower template concentrations, the amplification curves will begin to group together and C_T values will not fit the standard curve (Fig.2). The AppGreen 1-Step Extreme Low ROX kit is engineered to give rapid and accurate results from low template concentrations. If you observe grouping at higher template concentrations, try diluting the template. Alternatively, try AppGreen 1-Step Opti Low ROX Kit which is engineered for higher concentrations.



(Figure 1)



(Figure 2)

Denaturation*

Complete initial denaturation of the template cDNA is essential for efficient utilization of the template during the first amplification cycle – 2 min for cDNA.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mis-priming and non-specific PCR products. In order to have efficient amplification under fast cycling times, keep amplicon sizes between 80bp – 200bp and design primers so that they have a predicted melting temperature of ~60°C. (http://frodo.wi.mit.edu/primer3/).

Annealing

Incubation for 30s is usually sufficient. However, if non-specific PCR products are obtained in addition to the expected product, the annealing temperature should be optimized by increasing it stepwise by 1-2°C between 60-65°C – we do not recommend using annealing temperatures below 60°C.

TROUBLE SHOOTING / TECHNICAL SUPPORT

For troubleshooting please visit www.appletonwoods.co.uk/qPCRtroubleshooting.pdf for a trouble shooting guide on qPCR. If this does not resolve your issues, please email technicalsupport@appletonwoods.co.uk with details of your: amplicon size, reaction setup, cycling conditions, gel images.

ASSOCIATED PRODUCTS

Product	Pack Size	Product Code
Molecular Biology Grade Agarose	100g	AG002
Molecular Biology Grade Agarose	500g	AG001
Molecular biology grade water	100mL	BMW001
Molecular biology grade water	500mL	BMW002

More products available at www.appletonwoods.co.uk