For the Life Scientist

AppProbe Low ROX Mix (2X)

ORDERING INFORMATION

Description	Product Code	Pack Size
AppProbe Low ROX Mix (2X)	ARP302	200 reactions (2x 1mL)
	ARP303	500 reactions (5x 1mL)
	ARP305	5000 reactions (50x 1mL)

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

AppProbe Low ROX Mix (2X) is a high performance qPCR reagent which has been optimised for fast, specific and sensitive quantitative PCR using probe-detection technologies like Taqman, Molecular Beacons and Scorpion probes. It has pre-added and optimised $MgCl_2$ and dNTPs for highly reproducible qPCR. It contains a hot start polymerase which has been specifically engineered for highly specific qPCR and works in fast or standard thermal cycling conditions. It has been validated on various qPCR instruments - for a full list of compatible instruments see:

www.appletonwoods.co.uk/qPCRselectionguide.png

PROTOCOL

Prepare a qPCR master mix by mixing molecular biology grade water, AppProbe Low ROX Mix (2X), probes, and forward and reverse primers. Prepare sufficient master mix for the experimental sample number of reactions, DNA standard reactions and a no-template negative control (NTC). Aliquot the master mix into individual PCR tubes / wells and then add template DNA/ molecular biology grade water for NTC.

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
AppProbe Low ROX Mix (2X)	1X	10.0µL
Forward primer (10µM)	400nM	0.8µL
Reverse primer (10µM)	400nM	0.8µL
Probe (10µM)	200nM	0.4µL
Template DNA	100 – 1µg*	variable
Molecular Biology Grade water, (BMW001)		Up to 20µl final volume

For the Life Scientist

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

4. Perform qPCR using recommended thermal cycling conditions:

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

CONSIDERATIONS

Template DNA*

For optimal results, use between <1µg per reaction for genomic DNA, and for cDNA use <100ng in a 20µL reaction. Higher amount of template increases the risk of non-specific PCR products. Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

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 – 400bp (ideally not more than 200bp) and design primers so that they have a predicted melting temperature of ~60°C.

Denaturation*

Complete initial denaturation of the template DNA is essential for efficient utilization of the template during the first amplification cycle – use 2 min for cDNA and 3 min for genomic DNA.

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.

Optional steps

UDG pre-treatment. If carryover decontamination is required, include a 2 min UDG digestion step at 50°C before the initial denaturation step.

Melting curve analysis may be performed to verify the specificity and identity of the qPCR product. Primer-dimers may occur during qPCR if the primer design is not optimal. The primer dimers are distinguished from the specific product by a lower melting point.

Agarose gel electrophoresis of PCR products. When designing a new assay it is important to verify the PCR product specificity by gel electrophoresis, as melting temperatures of a specific product and primer-dimers may overlap depending on the sequence composition.

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
AxyPrep Mag PCR clean up Kit	5mL, 110 preps	AX401
Molecular biology grade water	500mL	BMW002

More products available at www.appletonwoods.co.uk