q•Probe 5X qPCR Mix with ROX, no ROX, with ROX vial, or capillary

for research and in vitro use only

Protocol

1. Thaw and briefly centrifuge all components
2. Prepare your reactions using the Recommended Reaction Mix
3. Briefly centrifuge your samples
4. Run qPCR using Recommended Cycling Settings

Recommended Reaction Mix

Component	Conc.	Amount	Final Conc.
q•Probe mix	5X	4 µL	1X
Primer, F[1]	10 pmol/µL	0.4 µL	200 nM
Primer, R[1]	10 pmol/µL	0.4 µL	200 nM
Probe[2]		variable	100 - 250 nM
Template DNA[3]		2 - 400 ng	0.1 - 20 ng/µL
Nuclease-free water		variable	
Total		20 µL	

1 To maximize assay sensitivity use lowest concentration possible without compromising reaction efficiency. Doubling the reverse primer concentration may improve performance. Further optimization can be tested using final concentrations of 100 - 400 nM.

2 Consult manufacturer recommendation for optimal probe concentration.

3 Template can be genomic DNA, plasmid DNA, or cDNA. For cDNA up to 10% of the final reaction may be cDNA (i.e. for a 20 µl reaction, use up to 2.0 µl of undiluted cDNA).

Recommended Cycling Settings

Cycle Step	Temp (ºC)	Time	Cycles
Initial denaturation	95	15 min	1
Denaturation	95	15 - 20 sec	40
Annealing & Extension	60	60 sec	

** Annealing temperature is 2 -6 ºC lower than T_M of primers.*

Estimating primer melting temperature:
For primers containing less than 25 nucleotides, T_M= 4 (G + C) + 2 (A + T), where G, C, A, T represent the number of respective nucleotides in the primer. If primers contain more than 25 nucleotides specialized software is recommended to calculate T_M.

q•Probe Order Information

Cat. No.	Desc.	Vol.	No. Rxns (20 µL each)	Mix Composition: q•Hot Taq	q•Probe 5X Buffer	Final $MgCl_2$ Conc.	dNTPs (dATP, dCTP, dGTP, & dTTP)	BSA	ROX*	Nuclease free H_2O
QPWR-01	with ROX	1 mL	250	✓	✓	1.5 mM	✓	No	Pre-mixed	4 mL
QPWR-02		2 mL	500							8 mL
QPWR-05		5 mL	1250							-
QPNR-01	no ROX	1 mL	250	✓	✓	1.5 mM	✓	No	No	4 mL
QPNR-02		2 mL	500							8 mL
QPNR-05		5 mL	1250							-
QPRV-01	with ROX vial	1 mL	250	✓	✓	1.5 mM	✓	No	50 µL	4 mL
QPRV-02		2 mL	500						100 µL	8 mL
QPRV-05		5 mL	1250						400 µL	-
QPC-01	capillary	1 mL	250	✓	✓	1.5 mM	✓	Yes	No	4 mL
QPC-02		2 mL	500							8 mL
QPC-05		5 mL	1250							-

** If volume is designated, then that volume of 0.1 mM ROX is in a separate vial. NOTE: ROX is a reference dye whose fluorescence provides a stable baseline that is used to normalize PCR generated fluorescent signals.*

Description

q•Probe qPCR mix is a ready-to-use cocktail for Probe-based quantitative real-time PCR. It contains all the components necessary for amplification and detection of DNA in qPCR: q•Hot Taq, q•Probe buffer, dNTPs, $MgCl_2$,stabilizers, and ROX dye if appropriate. Simply add nuclease-free water, template, primers, and probe.

Probe compatibility

- Fluorogenic probes, inlcuding hydrolysis probes (i.e. TaqMan®)
- Displacement probes (i.e. Molecular Beacon)
- Hybridization probes (i.e. LightCycler/ FRET probes)

Quality Control

q•Probe qPCR mix is DNase and RNase free. It is tested by functional assay using human genomic DNA.

Eco-friendly shipping & storage

- Shipped at ambient temperature without ice to reduce packaging waste
- Use within 2 weeks of arrival or store at -20°C

Some applications of this product are covered by patents issued to parties other than qARTA Bio, and may require a license which is not provided by the purchase of this product. User should obtain a patent license if appropriate.

User Protocol

T_+1-310-326-1542 E_technical.support@qartabio.com E_sales@qartabio.com W_www.qartabio.com

qARTA BIO