q•EvaGreen® 5X qPCR Mix with ROX, no ROX, ROX vial, capillary, or HRM

for research and in vitro use only

User Protocol

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•EvaGreen®	5x	4 µL	1x
Primer, F[1]	10 pmol/µL	0.16 - 0.5 µL	80 - 250 nM
Primer, R[1]	10 pmol/µL	0.16 - 0.5 µL	80 - 250 nM
Template DNA[2]		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 *Genomic DNA, plasmid DNA, or cDNA can be used as template. For cDNA up to 10% of the final reaction may be cDNA (i.e. for a 20 µl qPCR reaction, use up to 2.0 µl of undiluted cDNA).*

Recommended Cycling Settings

Cycle Step	Temp (°C)	Time	Cycles
Initial denaturation	95	15 min	1
Denaturation	95	15 sec	40
Annealing	T_M - 4[1]	20 sec	
Extension	72	20 sec	

Recommended Light Cycler 480 Settings

	Temp (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s) 96-well	Ramp Rate (°C/s) 384-well	Aquisition Rate (per °C)
Initial Denaturation	95	None	00:15:00	4.4	4.8	-
Amplification	95	None	00:00:10	4.4	4.8	15 sec
	T_M - 4[1]		00:00:15	2.2	2.5	-
	72	Single	E[2]	4.4	4.8	-
High Resolution Melt[3]	95	None	00:01:00	4.4	4.8	-
	40		00:01:00	2.2	2.5	-
	65		00:00:01	1	1	25
	95	Continuous	-	-	-	-
Cooling	40	Non	00:00:10	2.2	2.5	20 sec

[1] *Set annealing temperature to be 4°C lower than T_M of primers or set it to 60-65°C initially.*

[2] *Set elongation hold time E = Amplicon Length/15 (i.e. use 30 sec for a 450 bp amplicon).*

[3] *Use HRM cycle only for qARTA HRM mixes, else skip HRM settings.*

Estimating primer melting temperature:
For primers of 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. For longer primers, use specialized software to calculate T_M.

q•EvaGreen® Order Information

Cat. No.	Desc.	Vol.	No. Rxns 20 µL each	Mix Composition: q•Hot Taq	q•EvaGreen® 5X Buffer	Final $MgCl_2$ Conc.	dNTPs dATP, dCTP, dGTP, & dTTP	BSA	ROX*	Nuclease free H_2O
QGWR-01	with ROX	1 mL	250	✓	with EvaGreen® Dye	1.5 mM	✓	No	Pre-mixed	4 mL
QGWR-02		2 mL	500							8 mL
QGWR-05		5 mL	1250							-
QGNR-01	no ROX	1 mL	250						No	4 mL
QGNR-02		2 mL	500							8 mL
QGNR-05		5 mL	1250							-
QGRV-01	with ROX vial	1 mL	250						50 µL	4 mL
QGRV-02		2 mL	500						100 µL	8 mL
QGRV-05		5 mL	1250						200 µL	-
QGC-02	capillary	2 mL	500	✓	with EvaGreen®	1.5 mM	✓	Yes	No	8 mL
QGC-05		5 mL	1250							-
QGHWR-02	HRM with ROX	2 mL	500	✓	HRM Buffer with EvaGreen®	2.5 mM	✓	Yes	Pre-mixed	8 mL
QGHWR-05		5 mL	1250							-
QGHNR-02	HRM no ROX	2 mL	500						No	8 mL
QGHNR-05		5 mL	1250							-

** If volume is designated, then that volume of 0.1 mM ROX is in a separate vial.*

Description

q•EvaGreen® qPCR mix is a ready-to-use cocktail optimized containing all components necessary for amplification and detection of DNA in qPCR. Simply add nuclease-free water, template and primers.

- **HRM option**. HRM with ROX or without ROX mixes are optimized for High Resolution Melt.

EvaGreen® dye

EvaGreen® Dye is a green fluorescent nucleic acid dye replacing SYBR Green I, with added benefits:

- **Highly sensitive.** Exhibits lower PCR inhibition, which allows higher dye concentrations that in turn produce greater qPCR signals.
- **Nonmutagenic and noncytotoxic.** Impermeable to cell membranes.

Select the standard settings for SYBR Green or FAM to use q•EvaGreen mixes.

Eco-friendly shipping & storage

- Shipped at ambient temperature without ice, foam, or other wasteful packaging.
- Use within 2 weeks of arrival or store at -20°C.

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