

MiRXES

BlitzAmpHotstartqPCRMasterMixProtocol

For best results, keep all reagents on ice (or at 4°C) at all times during set up.

Real-time qPCR amplification and detection

- Step 1:** Gently thaw template DNA, primers and BlitzAmp (2x) on ice. Vortex and spin down reagents.
- Step 2:** If using cDNA templates directly from an RT reaction, dilute the template 1:10 in nuclease free water. Load template DNA into plate.
- Step 3:** Assemble qPCR reaction according to Table 1. Dispense appropriate volume of qPCR master mix per well.

Table 1 – Real-time qPCR setup, individual assays (96 well-plate)

Reagent	Volume
qPCR Master Mix (2x)	10 µl
qPCR Primers	(suggested final concentration 0.2 µM)
Nuclease-free water	to 20 µl
Template DNA	X µl
Total volume	20 µl

- Step 4:** Centrifuge the PCR plate briefly (30 s at 200 g).
- Step 5:** Perform Real-time PCR amplification with the following cycling parameters.

Table 2 – qPCR fast / lightning thermal cycling protocol

Cycles	Temperature	Time	Notes
1x	95°C	5 min	Polymerase activation
40x	95°C / 98 °C	10 s / 1s	Denaturation
	60°C / 60°C	30 s / 5s	Annealing/extension (acquire florescence reading at end of step) For amplicons >500bp, increase the annealing/extension time up to 1 min.

- Step 6:** Data analysis.

MiRXES BlitzAmp qPCR Master Mix

Real-time PCR Instrument	ROX requirement	BlitzAmp qPCR Master Mix (Rxn)
<ul style="list-style-type: none"> Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler® MiC qPCR 	Not required	1204202 (200) 1204204 (800) 1204205 (1200) 1204206 (1600)
<ul style="list-style-type: none"> Applied Biosystems® 7500, QuantStudio™, ViiA7™ Agilent Mx™ 	Low ROX	
<ul style="list-style-type: none"> Applied Biosystems® 7000, 7300, 7700, 7900HT, StepOne™, StepOnePlus™ 	High ROX	1204212 (200) 1204214 (800) 1204215 (1200) 1204216 (1600)

Product	Description	Cat. No.
BlitzAmp Hotstart qPCR Master Mix (200)	2 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 200 x 20 µl reactions	1204202
BlitzAmp Hotstart qPCR Master Mix (800)	8 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 800 x 20 µl reactions	1204204
BlitzAmp Hotstart qPCR Master Mix (1200)	12 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 1200 x 20 µl reactions	1204205
BlitzAmp Hotstart qPCR Master Mix (2400)	24 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 2400 x 20 µl reactions	1204206
BlitzAmp Hotstart qPCR Master Mix - HR (200)	2 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 200 x 20 µl reactions, For High-Rox qPCR instruments	1204212
BlitzAmp Hotstart qPCR Master Mix - HR (800)	8 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 800 x 20 µl reactions, For High-Rox qPCR instruments	1204214
BlitzAmp Hotstart qPCR Master Mix - HR (1200)	12 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 1200 x 20 µl reactions, For High-Rox qPCR instruments	1204215
BlitzAmp Hotstart qPCR Master Mix - HR (2400)	24 x 1,000 µl of BlitzAmp Hotstart qPCR Master Mix (2x), 2400 x 20 µl reactions, For High-Rox qPCR instruments	1204216

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