AMPLIQON
PCR ENZYMES & REAGENTS

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RealQ Fast 2x Master Mix Green

MADE IN DENMARK

Cat. No.	Reactions (25 µl)	RealQ Fast 2x Master Mix Green ID: 5030300	ROX Internal Reference Dye 200 µM ID: 5700300
A463403	500	5 x 1.25 ml	1 x 0.05 ml
A463404	1000	10 x 1.25 ml	1 x 0.05 ml
A463411	2500	25 x 1.25 ml	2 x 0.05 ml
A463412	5000	50 x 1.25 ml	4 x 0.05 ml
A463499	Sample - 40	1 x 0.5 ml	1 x 0.05 ml

Contents

RealQ Fast 2x Master Mix Green is a ready-to-use, optimized 2x master mix for dye-based real-time PCR. ROX is included for real-time instruments requiring an internal reference dye.

- RealQ Fast 2x Master Mix Green: optimized buffer system, 2Taq Hot Start DNA Polymerase, dNTPs, and fluorescent dye.
- 200 µM ROX internal reference dye.

Compatibility

All real-time PCR instruments with a FAM/SYBR filter (addition of ROX may be needed, see table 1).

Recommended Storage and Stability

Temperature	Duration
Room temperature	Up to 3 days
4° C	Up to 3 months
-20° C	Long term. See expiry on tube

Multiple freeze-thaw cycles should be avoided. Solutions containing fluorescent green DNA dye should be protected from light whenever possible.

ROX Reference Dye

ROX is used as passive reference dye in qPCR. The addition of ROX enables fluorescence normalization which helps to achieve a higher level of precision. The required concentration of ROX depends on the real-time PCR instrument – see table below.

Table 1: Recommended ROX concentrations for various real-time PCR instruments

Final ROX concentration	Real-time PCR instrument
0 nM	**Bio Molecular:** Mic qPCR Cycler **Bio-Rad:** iCyclerR iQ, iQ5 and MyiQ™, OpticonR, CFX 96, CFX 384 **Roche:** LightCyclerR 480, LightCycler 96, LightCyclerR 2.0, iCycler iQ System **Corbett:** Rotor-Gene™ 3000, 6000, Rotor-Gene Q **Eppendorf:** MasterCycler™ ep *realplex* **Cepheid:** Smart Cycler **MyGo:** Mini and Pro
30 nM	**Applied Biosystems:** ABI 7500 and ABI 7500 Fast, ABI ViiA7 **Agilent:** Mx3000™, Mx3005P™, and Mx4000™, Mx4000R, AriaMx
300 nM	**Applied Biosystems:** ABI 5700, 7000 PRISM, 7300, 7700, 7900, 7900HT and 7900HT Fast, StepOne™, StepOnePlus™

Protocol

Allow master mix to reach room temperature. Ensure sufficient mixing of the master mix (i.e. by vortexing) prior to reaction assembly.

1. Combine master mix, primers, template DNA and water according to table 2.

Table 2: Recommended reaction setup

Component	Vol./reaction	Final concentration*
RealQ Fast 2x Master Mix	12.5 µl	1x
Primer A (10 µM)	0.5 µl (0.2 – 1 µl)	0.2 µM (0.1 – 0.5 µM)**
Primer B (10 µM)	0.5 µl (0.2 – 1 µl)	0.2 µM (0.1 – 0.5 µM)**
200 µM ROX***	0.38 µl (1:100)	30 nM – Low ROX (Optional)
	0.38 µl (1:10)	300 nM – High ROX (Optional)
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 20 ng (1 – 100 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
Total volume**	25 µl	-

* Suggested starting conditions, optimisation range in parenthesis.
** Optimization of primer and probe concentrations is highly recommended.
*** If needed, prepare a fresh dilution of ROX internal reference dye.
For a final reaction concentration of 30 nM dilute 200 µM ROX 1:100 in PCR grade water. For a final reaction concentration of 300 nM dilute 200 µM ROX 1:10 in PCR grade water.
For a final reaction volume of 25 µl add 0.38 µl of the required ROX dilution.
The diluted ROX must be kept in a light-protected tube at 4°C.
**** Reaction volumes < 10 µl is not recommended. Smaller reaction volumes decrease signal intensity.

2. Mix the components thoroughly, then centrifuge to collect liquid at the bottom of the tube.
3. Transfer the appropriate volume to an optical plate or strip compatible with the chosen real-time PCR instrument. Seal the plate / strip.
4. Set up PCR program using one of the following settings:

Table 3: Standard 2-step cycling conditions.

	Step	Temperature	Duration	Cycle
STANDARD	Activate enzyme[a]	95°C	2 min	1
	Denature	95°C	15 sec	40
	Anneal/extend[b]	60°C	60 sec	

Table 4: Fast 2-step cycling conditions.

	Step	Temperature	Duration	Cycle
FAST	Activate enzyme[a]	95°C	2 min	1
	Denature	95°C	5 sec	40
	Anneal/extend[b]	60°C	30 sec	

Table 5: Super-fast 2-step cycling conditions. Sensitivity may be affected by fast cycling conditions.

	Step	Temperature	Duration	Cycle
SUPER-FAST	Activate enzyme[a]	95°C	2 min	1
	Denature	95°C	5 sec	40
	Anneal/extend[b]	60°C	15 sec	

[a] Can be reduced to 30 sec for non-complex templates.
[b] Choose an appropriate annealing temperature for the primer set used.

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Other product sizes, combinations and customized solutions are available. Please look at www.ampliqon.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

Issued 08/2025