

Manual

RT HS-PCR Mix SYBR® A/B/C

Ready-to-use mix for real-time Hot Start PCR with SYBR® Green. 2x concentrated.

catalog#	size
2017-100A / 2017-100B / 2017-100C	200 reactions in 25 μl
2017-1000A / 2017-1000B / 2017-1000C	2000 reactions in 25 μl

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.

The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Description

RT HS-PCR Mix SYBR® A, B or C are optimized ready to use real-time Hot Start PCR mixtures with SYBR® Green. Mixture contains all components required for qPCR except DNA template and primers.

This mix is recommended especially for difficult PCRs and multiplex PCR. StartWarm technology allows for activation of *Taq* DNA polymerase in the first steps of PCR. Long time of preliminary denaturation step is not required.

The premix formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible reaction.

Contents

	2017-100A/B/C	2017-1000A/B/C	storage
RT HS-PCR Mix SYBR® A, B or C	2 x 1.25 ml	20 x 1.25 ml	-20 °C, in darkness
Sterile water	2 x 1.5 ml	20 x 1.5 ml	-20 °C

RT HS-PCR Mix SYBR® A/B/C composition

component	amount
Taq DNA polymerase	0.1 U/μl
MgCl ₂	4 mM
dNTPs	0.5 mM of each dNTP
2x reaction buffer with SYBR® Green	

Notes

- Before use all solutions should be thawed thoroughly on ice, gently mixed by inverting the tube and briefly centrifuged.
- Up to 7x repeated freeze-thaw cycles do not influence the activity of this product.

Example PCR protocol

- 1. Thaw the RT HS-PCR Mix SYBR® A, B or C and sterile water on ice, gently mix by inverting the tubes and briefly centrifuge. Place the tubes on ice again.
- 2. Place PCR tubes on ice and add:

	PCR reaction volume		
component	10 µl	25 μΙ	50 µl
RT HS-PCR Mix SYBR® A, B or C	5 μΙ	12.5 µl	25 μΙ
Starter 1**	0.1 - 1 µM*	0.1 - 1 μM*	0.1 - 1 μM*
Starter 2**	0.1 - 1 µM*	0.1 - 1 μM*	0.1 - 1 μM*
DNA, cDNA template	10 pg - 1 μg	10 pg - 1 µg	10 pg - 1 µg
Sterile water	up to 10 μl	up to 25 μl	up to 50 μl

^{*} recommended for standard qPCR

- 3. Gently vortex the samples and briefly centrifuge to collect all droplets remaining on the tube walls and caps to the bottom of the tube.
- 4. Place the tubes in the thermocycler and start the PCR programme.

An example amplification profile:

step	temperature	time
Initial denaturation	95 ℃	2 - 3 min
25 - 45 cycles	95 °C 50 - 68 °C 72 °C	15 - 30 s 30 - 60 s 15 - 60 s*

^{*} depending on the length of PCR products

HiROX (0.6-1 μ l per 50 μ l of total reaction volume): Applied Biosystems: 7000, 7300, 7700, 7900HT Fast, StepOne, StepOnePlus.

LowROX (0.6-1 μ l per 50 μ l of total reaction volume): Applied Biosystems: 7500, Stratagene: Mx3000P, Mx3005P, Mx4000P.

^{**} final concentration in reaction mixture

PCR product melting analysis is recommended.

Recommended ROX mixture



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