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Manual

Sensitive RT HS-PCR Mix Probe 2x

High specificity ready-to-use mix for real-time hot-start PCR with fluorescent probe.
2x concentrated.

catalog #	size
2017-200PM	200 reactions in 25 µl
2017-2000PM	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

Description

Sensitive RT HS-PCR Mix Probe 2x is optimized for high specificity ready to use real-time hot-start PCR mixture for use with fluorescent probes. Mixture contains all components required for qPCR except DNA template, primers and probes. *Taq* DNA polymerase is blocked by monoclonal antibody.

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

	2017-200PM	**2017-2000PM**	**storage**
Sensitive Probe 2x	2 x 1.25 ml	20 x 1.25 ml	-20 °C
ultrapure water	2 x 1.5 ml	20 x 1.5 ml	-20 °C

Sensitive Probe 2x composition

component	**amount**
modified *Taq* DNA polymerase	0.1 U/µl
$MgCl_2$	10 mM
dNTPs	0.5 mM of each dNTP
2x reaction buffer	

Notes

- Before use all solutions should be thawed thoroughly on ice, gently mixed by inverting the tube and briefly centrifuged.
- Up to 3x repeated freeze-thaw cycles do not influence the activity of this product.

Example PCR protocol

1. Thaw **all components of the kit** 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 µl**	**50 µl**
Sensitive Probe 2x	5 µl	12.5 µl	25 µl
primer 1* **	0.1-1 µM*	0.1-1 µM*	0.1-1 µM*
primer 2* **	0.1-1 µM*	0.1-1 µM*	0.1-1 µM*
probe* **	0.05-0.1 µM**	0.05-0.1 µM**	0.05-0.1 µM**
DNA, cDNA template	10 pg-1 µg	10 pg-1 µg	10 pg-1 µg
ultrapure water	up to 10 µl	up to 25 µl	up to 50 µl

* recommended for standard qPCR
** amount of each probe should be optimized
*** final concentration in reaction mixture

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 °C	5 min
25 - 45 cycles **(2 step PCR)**	95 °C 58-70 °C*	15-30 s 15-60 s**

* depending on the elongation of the probe and primers temperature
** depending on the length of PCR products and/or number of amplicons in the tube

Recommended ROX mixture

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

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

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