A&A BIOTECHNOLOGY
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Manual

qPCR-HS Mix EvaGreen®

High specificity ready-to-use mix for real-time Hot Start PCR with EvaGreen®. Mixture contains monoclonal antibody blocked Taq DNA polymerase (RUN-HS). The product is recommended for High Resolution Melting (HRM) analysis.

catalog #	size
2008HS-100G	200 reactions in 25 µl
2008HS-1000G	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

qPCR-HS Mix EvaGreen® is optimized for high specificity ready to use real-time Hot Start PCR mixture with EvaGreen® dye for HRM technique. Mixture contains all components required for qPCR except DNA template and primers. Activation of the monoclonal antibody blocked RUN-HS polymerase occurs during initial denaturation in PCR. The product is recommended for High Resolution Melting (HRM) analyses.

Contents

	2008HS-100G		2008HS-1000G		
	quantity	**cat #**	**quantity**	**cat #**	**storage**
2x qPCR-HS Mix EvaGreen® (qPCR-HS Mix EG)	2 x 1.25 ml	K-28G-125A	20 x 1.25 ml	K-28G-125A	-20 °C
ultrapure water	2 x 1.5 ml	K-WUP-15A	20 x 1.5 ml	K-WUP-15A	-20 °C

Notes

- Before use, it is necessary to completely thaw and thoroughly mix the kit components by gently inverting the tube.
- Up to 7x repeated freeze-thaw cycles do not influence the activity of this product.

ROX reference dye

Some PCR instruments perform fluorescence signal correction and it is recommended to use ROX reference dye for signal normalization. Please follow manufacturer's instructions regarding addition of ROX reference dye and its concentration.

Example qPCR protocol

1. Add to the PCR tubes:

component	volume 25 µl	final concentration
2x qPCR-HS Mix EvaGreen®	12.5 µl	1X
primer 1 (10 µM)*	0.5 µl	0.2 µM
primer 2 (10 µM)*	0.5 µl	0.2 µM
DNA template	1-5 µl	< 250 ng/reakcja
ultrapure water	up to 25 µl	

*For optimization, a primer titration should be performed from 0,2 µM do 1 µM final concentration.

2. Gently mix the samples and briefly centrifuge.

3. Place the tubes in the thermocycler and start the PCR programme.
An example amplification profile:

reaction step	temperature	time	number of cycles
enzyme activation	95 °C	5 min	1
denaturation	95 °C	15 s	40
annealing*	50-68 °C	30 s	
extension**	72 °C	30 s	
melting step***	60-95 °C	0.05 s 0.2 °C	1

*Annealing temperature depends on primer sequence and the composition of the reaction mixture.
**Time of extension depends on the length of the amplicon.
***It is recommended to perform a melt curve to confirm the specificity of the reaction.

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