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.

# Example qPCR protocol

1. Add to the PCR tubes:

| component | volume<br>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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