SOLIS BIODYNE

Data Sheet

HOT FIREPol® EvaGreen® HRM Mix (no ROX), 5x

Cat. No.	Pack Size	20 µl rxn
08-31-0000S	0.2 ml	50
08-31-00001	1 ml	250
08-31-00001-5	5 x 1 ml	1250
08-31-00001-10	10 x 1 ml	2500
08-31-00020	20 ml	5000

For *in vitro* use only

Description:
HOT FIREPol® EvaGreen® HRM Mix (no ROX) is a 5x-concentrated ready-to-use solution for High Resolution Melt (HRM) Analysis, incorporating EvaGreen® dye. It comprises all the components necessary to perform qPCR and HRM Analysis: HOT FIREPol® DNA Polymerase, ultrapure dNTPs, $MgCl_2$ and EvaGreen® dye. The user simply needs to add water, template and primers.

HOT FIREPol® DNA Polymerase is activated by a 12 min incubation step at 95°C. This prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.

Applications:
- High Resolution Melt (HRM)

Mix Composition:
- **HOT FIREPol® DNA Polymerase**
- **5x EvaGreen® HRM buffer**
- **12.5 mM $MgCl_2$**
  1x PCR solution – 2.5 mM $MgCl_2$
- **dNTPs**
- **EvaGreen® dye**
- **BSA**

Shipping and Storage conditions:
Routine storage: -18ºC to -28ºC
Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of the product.

Manufactured by Solis BioDyne in compliance with the ISO 9001 and ISO 13485 certified Quality Management System.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Recommendation:
Reaction setup at room temperature.

Recommended qPCR reaction mix:

Component	Volume	Final conc.
HOT FIREPol® EvaGreen® HRM Mix (5x)	4 µl	1x
Forward primer (10 µM)	0.16–0.5 µl	80–250 nM
Reverse primer (10 µM)	0.16–0.5 µl	80–250 nM
DNA template	variable	variable[1]
H_2O PCR grade	up to 20 µl	
Total	**20 µl**	

[1] Conc. of cDNA 0.1 pg/µl–10 ng/µl; gDNA 10 pg/µl–4 ng/µl

Recommended qPCR cycling protocol:

Cycle step	Temp.	Time	Cycles
Initial activation[2]	**95ºC**	**12 min**	1
Denaturation	95ºC	15 s	40
Annealing[3]	60º–65ºC	20 s[4]	
Extension	72ºC	20 s[4]	

[2] To activate the polymerase, include an incubation step **at 95ºC for 12 minutes** at the beginning of the qPCR cycle.

[3] The annealing temperature (Ta) depends on the melting temperature (Tm) of the primers. A Ta that is about 2 to 5°C lower than the Tm of the primers is generally suitable. Performing temperature gradient is recommended.

[4] For templates longer than 150 bp, the annealing and extension time may be increased to 30 sec.

For HRM step please use settings recommended by the instrument manufacturer.

EvaGreen® Dye:
EvaGreen® is a DNA-binding dye with many features that make it superior for HRM. Apart from having similar spectra, EvaGreen® has three important features that set it apart from SYBR® Green I: EvaGreen® has much less PCR inhibition, is an extremely stable dye and has been shown to be non-mutagenic and non-cytotoxic. EvaGreen® is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM!

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Safety warnings and precautions:
This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water. Refer to Safety Data Sheet for more information.

Technical support:
Contact your sales representative for any questions or send an email to support@solisbiodyne.com

Online chat is available at www.solisbiodyne.com

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DS-08-31 v3
Revised 12.04.2022

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Trademark information: FIREPol® is an EU registered trademark of Solis BioDyne OÜ.

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