SOLIS BIODYNE

Data Sheet

HOT FIREPol® EvaGreen® qPCR Supermix, 5x

Cat. No.	Pack Size	20 µl rxn
08-36-0000S	0.2 ml	50
08-36-00001	1 ml	250
08-36-00001-5	5 x 1 ml	1250
08-36-00001-10	10 x 1 ml	2500
08-36-00020	20 ml	5000

For *in vitro* use only

Description:
HOT FIREPol® EvaGreen® qPCR Supermix is a 5x-concentrated ready-to-use solution for real time quantitative PCR assays, incorporating EvaGreen® dye. It comprises all the components necessary, excluding the template and primers, to perform highly sensitive qPCR.
HOT FIREPol® DNA Polymerase is activated by a 12 min incubation step at 95°C. The hot-start mechanism prevents the extension of non-specifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.

Applications:
- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Viral load determination

Mix Composition:
- **HOT FIREPol® DNA Polymerase**
- **Optimized buffer**
- **12.5 mM $MgCl_2$**
  1x PCR solution – 2.5 mM $MgCl_2$
- **dNTPs, including dUTP**
  The mix allows UNG treatment to prevent carryover contamination from previous PCR runs.
  IMPORTANT: UNG is not included in the HOT FIREPol® EvaGreen® Supermix and should be purchased separately.
- **EvaGreen® dye***
- **Internal reference based on ROX dye****
- **GC-enhancer**
- **Blue visualisation dye**

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.

Benefits:
- Highly specific and reproducible real time PCR
- Superior performance with GC-rich templates
- Blue visualisation dye for easy pipetting
- Excellent efficiency in case of low copy number targets
- UNG treatment capability due to dNTP blend of dUTP/dTTP
- Reaction set-up at room temperature
- Wide instrument compatibility independent of ROX requirement (except capillary)***.

Recommended qPCR reaction mix:

Component	Volume	Final conc.
HOT FIREPol® EvaGreen® qPCR Supermix (5x)	4 µl	1x
Forward primer (10 µM)	0.2–0.4 µl	100–200 nM
Reverse primer (10 µM)	0.2–0.4 µl	100–200 nM
OPTIONAL: UNG[1] (Uracil-N-glycosylase)	Variable	Variable[1]
DNA template	Variable	Variable[2]
H_2O PCR grade	up to 20 µl	
Total	**20 µl**	

[1] Please add UNG according to manufacturer's specification.

[2] 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
OPTIONAL: UNG treatment[3]	Variable[3]	Variable[3]	1
Initial activation[4]	**95ºC**	**12 min**	1
Denaturation	95ºC	15 s	40
Annealing[5]	60º–65ºC	20–30 s[6]	
Extension	72ºC	20–30 s[6]	

[3] **OPTIONAL!** Add UNG treatment step ONLY if UNG enzyme is added in the reaction mix for carryover contamination removal. Use UNG according to manufacturer's specification.

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

[5] 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.

[6] Use 20 sec for annealing and extension for templates shorter than 150 bp. For templates longer than 150 bp, the annealing and extension time may be increased to 30 sec.

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***EvaGreen® Dye:**
EvaGreen® is a DNA-binding dye with many features that make it a superior alternative to SYBR® Green I for qPCR. 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.

**Internal reference based on ROX dye is used to normalize the fluorescent reporter signal generated in qPCR. The product is compatible with low ROX system requirements

*****IMPORTANT UPDATE**!
HOT FIREPol® EvaGreen® qPCR Supermix is **not compatible with high ROX cyclers** such as Applied BioSystems® StepOne™ or StepOnePlus™.

With systems that require high ROX levels for signal normalization, we recommend using HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX), Cat. No. 08-24-00001

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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