Solis BioDyne
Data Sheet

5x HOT FIREPol® EvaGreen® qPCR Supermix

Suitable for ROX-dependent and ROX-independent qPCR cyclers

Cat. No.	Pack Size	Conc. ($MgCl_2$)
08-36-0000S	0.2 ml SAMPLE (50 reactions)	12.5 mM
08-36-00001	1 ml (250 reactions)	12.5 mM
08-36-00008	8 ml (2000 reactions)	12.5 mM
08-36-00020	20 ml (5000 reactions)	12.5 mM

For *in vitro* use only

Description:

5x HOT FIREPol® EvaGreen® qPCR Supermix is an optimised 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.

Benefits:

- **Wide instrument compatibility - suitable for qPCR cyclers regardless of ROX requirements (except capillary)**
- **Reaction set-up at room temperature**
- **Highly specific and reproducible real time PCR**
- **Excellent efficiency in case of low copy number targets**
- **UNG treatment capability due to dNTP blend of dUTP/dTTP**
- **Superior performance with long (up to 500 bp) and GC-rich templates**
- **Blue visualisation dye for easy pipetting**

Applications:

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

Shipping and Storage conditions:

Routine storage: -20ºC
Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of HOT FIREPol® EvaGreen® qPCR Supermix.

Mix Composition:

- **HOT FIREPol® DNA Polymerase**
- **Optimized buffer**
- **12.5 mM $MgCl_2$**
  1x PCR solution – 2.5 mM $MgCl_2$
- **dNTPs, including dUTP**
  Mix allows UNG treatment to prevent carryover contamination from previous runs.
  IMPORTANT: UNG is not included in the 5x HOT FIREPol® EvaGreen® Supermix
- **EvaGreen® dye**
- **Internal reference based on ROX dye**
- **GC-enhancer**
- **Blue visualisation dye**

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.

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.

Recommended qPCR reaction mix:

Component	Volume	Final conc.
5x HOT FIREPol® EvaGreen® qPCR Supermix	4 µl	1x
Primer Forward (10 pmol/µl)	0.2-0.4 µl	100-200 nM
Primer Reverse (10 pmol/µl)	0.2-0.4 µl	100-200 nM
OPTIONAL: UNG[1] (Uracil-N-lycosylase)	Variable[1]	Variable[1]
DNA template[2]	Variable[2]	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

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Recommended qPCR cycles:

Cycle step	Temp.	Time	Cycles
UNG treatment[3]	50ºC	2 min	1
Initial activation[4]	**95ºC**	**12 min**	1
Denaturation	95ºC	15 s	40
Annealing[5]	60º-65ºC	20 - 30 s	
Elongation[5]	72ºC	20 - 30 s	

[3] **OPTIONAL!** Add UNG treatment step ONLY if UNG enzyme is added in the reaction mix for carryover contamination removal

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

[5] Use 20 sec for annealing and elongation for templates shorter than 150 bp.