



## 5x HOT FIREPol® EvaGreen® qPCR Supermix

Suitable for ROX-dependent and ROX-independent qPCR cyclers

| Cat. No.    | Pack Size                       | Conc. (MgCl <sub>2</sub> ) |
|-------------|---------------------------------|----------------------------|
| 08-36-0000S | 0.2 ml SAMPLE<br>(50 reactions) | 12.5 mM                    |
| 08-36-00001 | 1 ml<br>(250 reactions)         | 12.5 mM                    |
| 08-36-00008 | 8 ml<br>(2000 reactions)        | 12.5 mM                    |
| 08-36-00020 | 20 ml<br>(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 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: -20°C

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<sub>2</sub>**  
*1x PCR solution – 2.5 mM MgCl<sub>2</sub>*
- **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®<br>EvaGreen® qPCR Supermix          | 4 µl                  | 1x                    |
| Primer Forward<br>(10 pmol/µl)                      | 0.2-0.4 µl            | 100-200 nM            |
| Primer Reverse<br>(10 pmol/µl)                      | 0.2-0.4 µl            | 100-200 nM            |
| OPTIONAL: UNG <sup>1</sup><br>(Uracil-N-lycosylase) | Variable <sup>1</sup> | Variable <sup>1</sup> |
| DNA template <sup>2</sup>                           | Variable <sup>2</sup> | Variable <sup>2</sup> |
| H <sub>2</sub> O PCR grade                          | up to 20 µl           |                       |
| <b>Total</b>  | <b>20 µl</b>          |                       |

<sup>1</sup> Please add UNG according to manufacturer's specification.

<sup>2</sup> Conc. of cDNA 0.1 pg/µl -10 ng/µl ; gDNA 10 pg/µl – 4 ng/µl

### Recommended qPCR cycles:

| Cycle step                            | Temp.       | Time          | Cycles |
|---------------------------------------|-------------|---------------|--------|
| UNG treatment <sup>3</sup>            | 50°C        | 2 min         | 1      |
| <b>Initial activation<sup>4</sup></b> | <b>95°C</b> | <b>12 min</b> | 1      |
| Denaturation                          | 95°C        | 15 s          | 40     |
| Annealing <sup>5</sup>                | 60°-65°C    | 20 - 30 s     |        |
| Elongation <sup>5</sup>               | 72°C        | 20 - 30 s     |        |

<sup>3</sup> **OPTIONAL!** Add UNG treatment step **ONLY** if UNG enzyme is added in the reaction mix for carryover contamination removal

<sup>4</sup> To activate the polymerase, include an incubation step **at 95°C for 12 minutes** at the beginning of the qPCR cycle.

<sup>5</sup> Use 20 sec for annealing and elongation for templates shorter than 150 bp.

**Permitted Use:** This product is supplied for research use only (the **Permitted Use**). If the customer wishes to use the product for any purpose other than the Permitted Use, including (without limitation) resale or alteration, the customer should obtain the appropriate licence from Solis BioDyne. Some applications of this product may require a license/licenses from one or more third parties which are not provided by the purchase of this product. Users should obtain the licence if required. Covered by the patent EP2501716, made by the methods of US Patent No 9,321,999.

**Warranty and Disclaimer:** This product shall comply with its relevant specification and be fit for its stated purpose, but Solis BioDyne gives no other warranty and makes no representation as to description or quality. Any such warranty or representation is excluded, to the fullest extent permitted by law. In particular, but without limiting the foregoing, Solis BioDyne shall not be liable for the failure of the product to comply with its relevant specification where such failure arises as a result of: (i) customer negligence or because the customer failed to follow any of the applicable technical data or safety sheets, standard user materials, use guidelines or any other information provided by Solis BioDyne as to the storage, transportation, handling, use or maintenance of the products or other good practice regarding the same, or (ii) the customer altering the products in any way without the prior written consent from Solis BioDyne, or (iii) the products differing from the relevant specification as a result of changes made to ensure their compliance with applicable statutory or regulatory requirements.

Nothing shall limit or exclude Solis BioDyne's liability for death or personal injury caused by its negligence, fraud or fraudulent misrepresentation or any matter in respect of which it would be unlawful for Solis BioDyne to exclude or restrict liability. Without limiting the foregoing, Solis BioDyne shall under no circumstances whatever be liable to the customer, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of profit, or any indirect or consequential loss arising under or in connection with the products and Solis BioDyne's total liability to the customer in respect of all other losses arising under or in connection with the product, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed the price of the products supplied in respect of which the liability has arisen.

FIREPol® is a trademark of Solis BioDyne. SYBR® is a registered trademark of Molecular Probes, Inc.

EvaGreen® is a registered trademark of BIOTIUM, INC. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer, where such research does not include testing, analysis or screening services for any third party in return for compensation on a per test basis. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.