

Data Sheet

HOT FIREPoI[®] Multiplex qPCR Mix (ROX), 5x

| Cat. No. | Pack Size | 20 µl rxn |
|----------------|-----------|-----------|
| 08-02-0000S | 0.2 ml | 50 |
| 08-02-00001 | 1 ml | 250 |
| 08-02-00001-5 | 5 x 1 ml | 1250 |
| 08-02-00001-10 | 10 x 1 ml | 2500 |
| 08-02-00008 | 8 ml | 2000 |
| 08-02-00020 | 20 ml | 5000 |

For *in vitro* use only

Description:

HOT FIREPoI[®] Multiplex qPCR Mix is optimized for amplifying up to 4 targets in a single reaction in real-time quantitative PCR assays. The qPCR Mix comprises all the components necessary (except primers, probes and template) to perform qPCR: HOT FIREPoI[®] DNA Polymerase, optimized buffer components, ultrapure dNTPs and MgCl₂ and ROX passive reference dye according to system requirements.

HOT FIREPoI[®] Multiplex qPCR Mix (ROX) is optimized for DNA hydrolysis probes based on the 5' flap endonuclease activity.

HOT FIREPoI[®] DNA Polymerase is activated by a 10 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:

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

Mix Composition:

- **HOT FIREPoI[®] DNA Polymerase**
- **5x Multiplex qPCR buffer**
- **15 mM MgCl₂**
1x PCR solution – 3 mM MgCl₂
- **dNTPs**, including dUTP
The mix allows UNG treatment to prevent carryover contamination from previous runs.
IMPORTANT: UNG is not included in the HOT FIREPoI[®] Multiplex qPCR Mix and should be purchased separately.
- **Internal reference based on ROX dye**
The dye is used to normalize the fluorescent reporter signal generated in qPCR. The product is compatible with both low ROX and high ROX system requirements.
If ROX, Texas Red or similar dye is used as one of the fluorophores, ROX passive reference dye might interfere with the signal. A version without passive reference or with Purple reference dye is available.

Shipping and Storage conditions:

Routine storage: -18°C to -28°C

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.

Recommendations:

Reaction setup at room temperature.

In order to prevent contamination, we recommend you to setup the reaction under laminar or in PCR box.

Recommended qPCR reaction mix:

| Component | Volume | Final conc. |
|--|--------------|-----------------------|
| HOT FIREPoI [®] Multiplex qPCR Mix (ROX) (5x) | 4 µl | 1x |
| Forward primer (10 µM) | 0.4–0.8 µl | 200–400 nM (each) |
| Reverse primer (10 µM) | 0.4–0.8 µl | 200–400 nM (each) |
| Probe | x µl | 100–250 nM (each) |
| OPTIONAL: UNG (Uracil-N-glycosylase) | Variable | Variable ¹ |
| DNA template | Variable | Variable ² |
| H ₂ O 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 | Cycle s |
|---------------------------------------|-----------------------|-----------------------|---------|
| OPTIONAL: UNG treatment ³ | Variable ³ | Variable ³ | 1 |
| Initial activation³ | 95°C | 10 min | 1 |
| Denaturation | 95°C | 15–20 s | 40 |
| Annealing/Extension ⁴ | 60°C | 60 s | |

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.

³ To activate the polymerase, include an incubation step **at 95°C for 10 minutes** at the beginning of the qPCR cycle.

⁴ The annealing temperature (T_a) depends on the melting temperature (T_m) of the primers. A T_a that is about 2 to 5°C lower than the T_m of the primers is generally suitable. Performing temperature gradient is recommended.

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

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