HOT FIREPol® GC Master Mix Kit

Catalogue Number	Pack Size	20 µl rxn
04-43-00S15	0.1 ml	25
04-43-00115	1 ml	250
04-43-00115-5	5 x 1ml	1250
04-43-02015	20 ml	5000

Store at –20°C upon receipt

Shipping:

At room temperature

Batch Number and Expiry Date:

See vial

Storage and Stability*:

- Routine storage at –20°C (–28°C to –18°C) until expiry date.
- Stable at 4°C (2°C to 8°C) for 6 months.
- Stable at room temperature (25°C) for 1 month.
- Freeze-thaw stability: 30 cycles

Reaction setup:

At room temperature.

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

Product description:

- HOT FIREPol® GC Master Mix Kit has been developed for working with difficult GC-rich templates and DNA secondary structures. It shows excellent amplification with templates up to 79% GC content.
- This kit includes a 5x-concentrated ready-to-use Master Mix containing all the reagents required for PCR (except template, primers and water). The special buffer of the 5x Master Mix is optimized for high yield GC-rich amplification.
- 100% DMSO and 25 mM $MgCl_2$ are included in the package in separate vials for increased flexibility.

Kit content:

Component	Catalogue Number			
	04-43-00S15	04-43-00115	04-43-00115-5	04-33-02015
HOT FIREPol® GC Master Mix (5x)	0.1 ml	1 ml	5 x 1 ml	20 ml
100% DMSO	0.1 ml	0.5 ml	5 x 0.5 ml	10 ml
25 mM $MgCl_2$	0.1 ml	0.5 ml	5 x 0.5 ml	20 x 0.5 ml

HOT FIREPol® GC Master Mix (5x) contains:

- HOT FIREPol® DNA Polymerase – chemically modified FIREPol® DNA Polymerase enabling hot-start activation.
- 5x HOT FIREPol® GC Buffer
- 7.5 mM $MgCl_2$ – 1.5 mM $MgCl_2$ in 1x PCR solution
- dNTPs
- BSA

Additional reagents required:

- Template DNA
- Gene-specific primer pair
- Nuclease-free PCR Grade Water (Cat. No. water-025)

Step-by-step guidelines:

1. Thaw the reagents at room temperature. Mix each reagent by gentle vortexing or pipetting up and down, then spin down.

2. Prepare a reaction mix at room temperature. Add all required components except the template DNA.

Component	Volume[1]	Final conc.
HOT FIREPol® GC Master Mix (5x)	4 µl	1x
25 mM $MgCl_2$[2]	As required	As required
Forward primer (10 µM)	0.2-0.6 µl	0.1-0.3 µM
Reverse primer (10 µM)	0.2-0.6 µl	0.1-0.3 µM
OPTIONAL: 100% DMSO[3]	As required	Up to 10%
Template DNA	Variable	Variable[4]
Nuclease-free water	up to 20 µl	
Total reaction volume	**20 µl**	

[1] Scale all components proportionally according to sample number and reaction volumes. Make sure you use enough of each reagent for your reactions, plus 10% extra volume to accommodate pipetting errors.

[2] HOT FIREPol® GC Master Mix (5x) contains 1.5 mM $MgCl_2$ at 1X. Additional $MgCl_2$ may be added separately if required.

[3] DMSO is recommended as a PCR additive for templates with high GC content. In some cases, DMSO is also required to relax secondary structures. While testing it is recommended to include one sample with additional 2.5 % DMSO to test if it improves the results. For further DMSO optimization the concentration can be raised in 2.5% increments up to 10% based on the table below.

[4] Conc. of cDNA 0.01 pg/µl–0.1 ng/µl; gDNA 0.1 ng/µl–50 ng/µl.

Final $MgCl_2$ concentration	1.75 mM	2 mM	2.5 mM
Additional volume of 25 mM $MgCl_2$	0.2 µl	0.4 µl	0.8 µl

Final DMSO concentration	2.5 %	5 %	7.5 %	10 %
Additional volume of 25 mM $MgCl_2$	0.5 µl	1 µl	1.5 µl	2 µl

3. Mix the reaction mix thoroughly, then spin down. Dispense appropriate volumes of mix into PCR wells or tubes.

4. Add template DNA to the PCR wells. Seal the wells using the procedure recommended for the cycling instrument being used, and centrifuge the reactions briefly.

5. Incubate your PCR reactions in thermal cycler as follows.

Step	Temperature	Time	Cycles
Initial denaturation[1]	95°C	12 min	1
Denaturation	95°C	15–30 sec	25–30
Annealing[2]	54–66°C	30–60 sec	
Extension[3]	72°C	1.5 min–5.5 min	
Final extension	72°C	5–10 min	1

[1] To activate the polymerase it is essential to include an incubation step at **95°C for 12 minutes** at the beginning of the PCR cycle.

[2] The annealing temperature depends on the melting temperature of the primers.

[3] Extension time depends on the length of the fragment to be amplified. A time of 1 min/kb is recommended.

Unit defintion:

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 74°C.

Safety precautions:

Please refer to the Safety Data Sheet for more information.

Technical support:

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

DS-04-43 v1. Effective from: 30.01.2024

Reason for revision: product name and catalogue number updated: former HOT FIREPol® GC Master Mix (5x) (catalogue no. 04-33-00S15, 04-33-00115, 04-33-00115-5, 04-33-02015).

*Product stability is assessed using routine QC assays and QC criteria set forth in the product specification and are intended to provide guidelines for shipping and storage conditions only. The customer or its designee shall be responsible for conducting all necessary stability testing applicable to their assay and/or QC criteria, and to comply with any applicable regulatory requirements or guidelines. Such stability testing shall include testing to validate the lead times for shipment, the shelf life of, and the product specifications applicable to shipment, storage and handling of the assay assembled and packed by the customer.

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Trademark information: FIREPol is a registered trademark of Solis BioDyne OÜ

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