SOLIS BIODYNE

Data Sheet

HOT FIREPol® GC Master Mix, 5x

Cat. No.	Pack Size	20 µl rxn
04-33-00S15	0.1 ml	25
04-33-00115	1 ml	250
04-33-00115-5	5 x 1 ml	1250
04-33-00115-10	10 x 1 ml	2500
04-33-02015	20 ml	5000

For *in vitro* use only

Description:

HOT FIREPol® GC Master Mix is designed to provide highly specific high-yield amplification of GC-rich templates. Master Mix is a 5x-concentrated ready-to-use solution containing all reagents required for PCR (except template, primers and water). 100% DMSO and 25 mM $MgCl_2$ are included in the package in separate vials.

Applications:

- Hot Start GC-rich PCR
- Fragment analysis
- TA cloning

Mix Composition:

- **HOT FIREPol® DNA polymerase**
- **5x HOT FIREPol® GC Buffer**
- **7.5 mM $MgCl_2$**
  1x PCR solution – 1.5 mM $MgCl_2$
- **dNTPs**
- **BSA**

Reagents provided with the mix in separate vials:

- **100% DMSO**
- **25 mM $MgCl_2$**

Shipping and Storage conditions:

Routine storage: -18ºC to -28ºC
Shipping and temporary storage for up to 1 month at room temperature or storage for up to 6 months at 2–8°C 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.

Recommended PCR reaction mix:

Component	Volume	Final conc.
HOT FIREPol® GC Master Mix (5x)	4 µl	1x
25 mM $MgCl_2$[1]	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[2]	As required	Up to 10%
Template DNA	Variable	Variable[3]
H_2O	Up to 20 µl	

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

[2] 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 following table.

[3] 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 100% DMSO	0.5 µl	1 µl	1.5 µl	2 µl

Recommended PCR cycling protocol:

Operation	Temp.	Time	Cycles
Initial activation[4]	**95°C**	**12 min**	1
Denaturation	95°C	30 s	25–30
Annealing[5]	54–66°C	30–60 s	
Extension[6]	72°C	1.5 min–5.5 min	
Final extension	72°C	5 min	

[4] To activate the polymerase, include an incubation step **at 95ºC for 12 minutes** at the beginning of the PCR 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] Extension time depends on the length of the fragment to be amplified. A time of 1 min/kb is recommended.

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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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