Solis BioDyne
Data Sheet

5x HOT FIREPol® GC Master Mix

Cat. No.	Pack Size	Conc. ($MgCl_2$)
04-33-00S15	0.1 ml SAMPLE (25 reactions)	7.5 mM
04-33-00115	1 ml (250 reactions)	7.5 mM
04-33-02015	20 ml (5000 reactions)	7.5 mM

For *in vitro* use only

Description:

5x HOT FIREPol® GC Master Mix is designed to provide highly specific high-yield amplification of GC-rich templates. Master Mix is a premixed 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**

In separate vials:

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

Shipping and Storage conditions:

Routine storage: -20°C
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 5x HOT FIREPol® GC Master Mix.

Recommendations:

Reaction setup at room temperature is highly recommended for HOT FIREPol® GC Master Mix.

Recommended PCR reaction mix:

Component	Volume	Final conc.
5x HOT FIREPol® GC Master Mix	4 µl	1 x
25 mM $MgCl_2$[1]	As required	As required
Forward primer (10 pmol/µl)	0.2-0.6 µl	0.1-0.3 µM
Reverse primer (10 pmol/µl)	0.2-0.6 µl	0.1-0.3 µM
OPTIONAL: 100% DMSO[2]	As required	Up to 10%
Template DNA[3]	Variable[2]	Variable[2]
H_2O	Up to 20 µl	

[1]5x HOT FIREPol® GC Master Mix 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 cycles:

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

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

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.

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