

## HOT FIREPoI® Blend Master Mix Ready to Load With 12.5 mM MgCl<sub>2</sub>, 5x

Cat. No.	Pack Size	20 µl rxn
04-25-00S25	0.1 ml	25
04-25-00125	1 ml	250
04-25-00125-5	5 x 1 ml	1250
04-25-00125-10	10 x 1 ml	2500
04-25-02025	20 ml	5000

For *in vitro* use only

### Description:

HOT FIREPoI® Blend Master Mix Ready to Load is a 5x-concentrated ready-to-use solution containing all reagents required for PCR (except template, primers and water), additional compound needed for direct loading onto agarose gel and two tracking dyes (blue and yellow) that allow to monitor progress during electrophoresis.

HOT FIREPoI® Blend Master Mix contains two carefully optimized enzymes – HOT FIREPoI® DNA polymerase and a proofreading polymerase. This enzyme blend has both the 5' flap endonuclease activity as well as the 3'→5' proofreading activity. HOT FIREPoI® Blend Master Mix exhibits an increased fidelity (up to five-fold) compared to HOT FIREPoI®. Generated PCR products are compatible with blunt-end and TA cloning procedures (to increase the blunt end cloning efficiency treat the PCR products with T4 DNA polymerase or DNA polymerase I large Klenow fragment prior to cloning).

### Applications:

- Hot Start PCR

### Mix Composition:

- **HOT FIREPoI® DNA polymerase**
- **Proofreading enzyme**
- **5x Blend Master Mix Buffer**
- **12.5 mM MgCl<sub>2</sub>**  
*1x PCR solution – 2.5 mM MgCl<sub>2</sub>*
- **1 mM dNTPs of each**  
*1x PCR solution – 200 µM dATP, 200 µM dCTP, 200 µM dGTP and 200 µM dTTP*
- **BSA**
- **Blue dye**  
*Migration equivalent to 3.5–4.5 kb DNA fragment*
- **Yellow dye**  
*Migration equivalent to 35–45 bp DNA fragment*
- **Compound that increases sample density for direct loading**

### Shipping and Storage conditions:

Routine storage: -18°C to -28°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 the product.

### Recommendations:

Reaction setup at room temperature.

We recommend using HOT FIREPoI® Blend Master Mix Ready to Load (5x) in any PCR application that will be visualized by agarose gel electrophoresis and ethidium bromide staining. HOT FIREPoI® Blend Master Mix Ready to Load (5x) is not recommended for use in applications where spectro-photometric measurements (absorbance or fluorescence) are necessary because yellow and blue dyes can interfere with these applications.

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

### Recommended PCR reaction mix:

Component	Volume	Final conc.
HOT FIREPoI® Blend Master Mix Ready to Load (5x)	4 µl	1x
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
DNA template	variable	variable <sup>1</sup>
H <sub>2</sub> O	Up to 20 µl	

<sup>1</sup> Conc. of cDNA 0.01 pg/µl–0.1 ng/µl ; gDNA 0.1 ng/µl–10 ng/µl

### Recommended PCR cycling protocol:

Operation	Temp.	Time	Cycles
<b>Initial activation<sup>2</sup></b>	<b>95°C</b>	<b>12–15 min</b>	<b>1</b>
Denaturation	95°C	10–20 s	25–30
Annealing <sup>3</sup>	54–66°C	30–0 s	
Elongation <sup>4</sup>	72°C	20 s–4 min	
Final elongation	72°C	5–10 min	

<sup>2</sup> To activate the polymerase, include an incubation step **at 95°C for 12–15 minutes** at the beginning of the PCR cycle.

<sup>3</sup> The annealing temperature (T<sub>a</sub>) depends on the melting temperature (T<sub>m</sub>) of the primers. A T<sub>a</sub> that is about 2 to 5°C lower than the T<sub>m</sub> of the primers is generally suitable. Performing temperature gradient is recommended.

<sup>4</sup> Extension time depends on the length of the fragment to be amplified. A time of 1 min/kb is recommended.

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.**

**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](mailto:support@solisbiodyne.com)

Online chat is available at [www.solisbiodyne.com](http://www.solisbiodyne.com)

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