



5x HOT FIREPol® Blend Master Mix Ready to Load

With 7.5 mM MgCl₂

Cat. No.	Pack Size	Conc. (MgCl ₂)
04-25-00S15	0.1 ml SAMPLE (25 reactions)	7.5 mM
04-25-00115	1 ml (250 reactions)	7.5 mM
04-25-02015	20 ml (5000 reactions)	7.5 mM

For *in vitro* use only

Description:

5x HOT FIREPol® Blend Master Mix Ready to Load is a premixed 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 FIREPol® Blend Master Mix contains two carefully optimized enzymes – HOT FIREPol® 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 FIREPol® Blend Master Mix exhibits an increased fidelity (up to five fold) compared to HOT FIREPol®. 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 FIREPol® DNA polymerase**
- **Proofreading enzyme**
- **5x Blend Master Mix Buffer**
- **7.5 mM MgCl₂**
1x PCR solution – 1.5 mM MgCl₂
- **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: -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® Blend Master Mix Ready to Load.

Recommendations:

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

We recommend using 5x HOT FIREPol® Blend Master Mix Ready to Load in any PCR application that will be visualized by agarose gel electrophoresis and ethidium bromide staining. 5x HOT FIREPol® Blend Master Mix Ready to Load 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.

Recommended PCR reaction mix:

Component	Volume	Final conc.
5x HOT FIREPol® Blend Master Mix Ready to Load	4 μl	1 x
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
DNA template ¹	variable ¹	variable ¹
H ₂ O	Up to 20 μl	

¹Conc. of cDNA 0.01 pg/μl -0.1 ng/μl ; gDNA 0.1 ng/μl – 10 ng/μl

Recommended PCR cycles:

Operation	Temp.	Time	Cycles
Initial activation²	95°C	12-15 min	1
Denaturation	95°C	10-20 s	25-30
Annealing	54-66°C	30-60 s	
Elongation	72°C	20 s - 4 min	
Final elongation	72°C	5-10 min	

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

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

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.

Permitted Use: This product is supplied for research use only (the **Permitted Use**). If the customer wishes to use the product for any purpose other than the Permitted Use, including (without limitation) resale or alteration, the customer should obtain the appropriate licence from Solis BioDyne. Some applications of this product may require a license/licenses from one or more third parties which are not provided by the purchase of this product. Users should obtain the licence if required. Covered by the patent EP2501716, made by the methods of US Patent No 9,321,999.

Warranty and Disclaimer: This product shall comply with its relevant specification and be fit for its stated purpose, but Solis BioDyne gives no other warranty and makes no representation as to description or quality. Any such warranty or representation is excluded, to the fullest extent permitted by law. In particular, but without limiting the foregoing, Solis BioDyne shall not be liable for the failure of the product to comply with its relevant specification where such failure arises as a result of: (i) customer negligence or because the customer failed to follow any of the applicable technical data or safety sheets, standard user materials, use guidelines or any other information provided by Solis BioDyne as to the storage, transportation, handling, use or maintenance of the products or other good practice regarding the same, or (ii) the customer altering the products in any way without the prior written consent from Solis BioDyne, or (iii) the products differing from the relevant specification as a result of changes made to ensure their compliance with applicable statutory or regulatory requirements.

Nothing shall limit or exclude Solis BioDyne's liability for death or personal injury caused by its negligence, fraud or fraudulent misrepresentation or any matter in respect of which it would be unlawful for Solis BioDyne to exclude or restrict liability. Without limiting the foregoing, Solis BioDyne shall under no circumstances whatever be liable to the customer, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of profit, or any indirect or consequential loss arising under or in connection with the products and Solis BioDyne's total liability to the customer in respect of all other losses arising under or in connection with the product, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed the price of the products supplied in respect of which the liability has arisen.