

Data Sheet

HOT FIREPoI® MultiPlex Mix Ready to Load with 10 mM MgCl₂, 5x

Cat. No.	Pack Size	20 µl rxn
04-36-00S20	0,1 ml	25
04-36-00120	1 ml	250
04-36-00120-5	5 x 1 ml	1250
04-36-00120-10	10 x 1 ml	2500
04-36-02020	20 ml	5000

For *in vitro* use only

Description:

HOT FIREPoI® MultiPlex Mix Ready to Load is a 5x-concentrated ready-to-use solution containing all reagents required for hot-start multiplex PCR (except template, primers and water). The product is optimized for amplification of up to 18 targets in a single reaction and it contains an additional compound needed for direct loading onto agarose gel and two tracking dyes (blue and yellow) that allow to monitor progress during electrophoresis. The product

Applications:

- Hot Start PCR
- Multiplex PCR

Mix Composition:

- **HOT FIREPoI® DNA polymerase**
- **5x MultiPlex Buffer**
- **10 mM MgCl₂**
1x PCR solution – 2mM MgCl₂
- **dNTPs**
- **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.

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 is highly recommended for HOT FIREPoI® MultiPlex Mix. HOT FIREPoI® MultiPlex Mix Ready to Load is not recommended for use in applications where spectrophotometric measurements (absorbance or fluorescence) are necessary because yellow and blue dyes can interfere with these applications.

Recommended PCR reaction mix:

Component	Volume	Final conc.
HOT FIREPoI® MultiPlex Mix Ready to Load (5x)	4 µl	1x
Forward primer (10 µM)	0.2–0.6µl	0.1–0.3 µM (each)
Reverse primer (10 µM)	0.2–0.6µl	0.1–0.3 µM (each)
Template DNA	Variable	Variable ¹
H ₂ O	Up to 20 µl	

¹ 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
Initial activation²	95°C	12 min	1
Denaturation	95°C	20–30 s	25–30
Annealing ³	58–62°C	30–60 s	
Elongation ⁴	72°C	30 s–3 min	
Final elongation	72°C	5–10 min	1

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

³ The annealing temperature (T_a) depends on the melting temperature (T_m) of the primers. A T_a that is about 2 to 5°C lower than the T_m of the primers is generally suitable. Performing temperature gradient is recommended.

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

Troubleshooting:**For troubleshooting the following additives may be included with your order free of charge upon request:**

- **25 mM MgCl₂** (cat. no. 05-11-00025) can be used for optimization of the MgCl₂ concentration. The 1x PCR mix solution of 2 mM MgCl₂ gives satisfactory results in most applications. If necessary, the MgCl₂ concentration may be increased in 0.25-1 mM increments up to 5 mM concentration.
- **10x GC-rich Enhancer** (cat. no. 05-16-00010) can be used for optimization with GC-rich templates. It modifies the melting behavior of nucleic acids and enhances the amplification of regions with secondary structures and high GC-content. 10x GC-rich Enhancer should be used at a defined working concentration (1x, 2x or 3x solution) and only if non-specific amplification occurs.
- **100% DMSO** is recommended as a PCR additive for templates with high GC content. In some cases, DMSO is also required to relax secondary structures. For optimization DMSO concentration can be raised in 2.5% increments up to 10%.

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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