Product Testing Manual

5x HOT FIREPol® Probe Universal qPCR Mix

Kit Contents		
	CAT. NO.	VIAL SIZE
5x HOT FIREPol® Probe Universal qPCR Mix	08-17-0000S	0.2
100% DMSO	DMSO_0.1	0.1

Storage

Routine storage: -20°C

Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of 5x HOT FIREPol® Probe Universal qPCR Mix

Instrument Compatibility

5x HOT FIREPol® Probe Universal qPCR Mix is is designed for use with standard cycling mode on standard and fast qPCR platforms regardless of requirements in ROX. 5x HOT FIREPol® Probe Universal qPCR Mix contains reference dye based on ROX that exhibits sufficient fluorescence for data normalization for all qPCR cyclers from Applied Biosystems and Stratagene. The presence of reference dye does not interfere with qPCR on any other platform.

Important notes:

- 5x HOT FIREPol® Probe Universal qPCR Mix is optimized for quantification of wide range of gDNA and cDNA targets using dual-labelled DNA/LNA hydrolysis probes based on the 5´–3´ exonuclease activity.
- Product is ambient temperature stable thanks to Stability-TAG Technology. Product can be shipped and reaction set-up can be done at room temperature.
- Start PCR cycling with an initial activation step of 10 min at 95°C to activate HOT FIREPol® DNA polymerase.
- Always follow the cycling conditions specified in this protocol. 5x HOT FIREPol® Probe Universal qPCR Mix does not support Fast Mode thermal cycling conditions. When using on Fast instruments, use standard mode thermal cycling conditions.
- Concentration of the 5x HOT FIREPol® Probe Universal qPCR Mix is 5x.
- Preparation of a reaction master mix, which includes all reaction components except template DNA, helps avoiding of pipetting errors and is recommended.

Description

5x HOT FIREPol® Probe Universal qPCR Mix is optimized for real-time quantitative PCR assays and contains all the components necessary to perform singleplex or duplex qPCR, with the exception of template, primers, and probes. The qPCR Mix contains optimized components and HOT FIREPol® DNA Polymerase supplied in a proprietary reaction buffer that enables efficient amplification of regular and GC-rich targets.

HOT FIREPol® DNA Polymerase is activated by a 10 min incubation step at 95°C. This prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.

HOT FIREPol® Probe Universal qPCR buffer is optimized for qPCR analysis using dual-labelled DNA/LNA hydrolysis probes based on the 5´–3´ exonuclease activity. The buffer composition allows efficient qPCR amplification for wide range of targets including AT-rich, regular and GC rich targets.

dNTPs including dUTP. dUTPs are included in the mix partially substituting dTTP-s, allowing UNG-treatment between reactions to prevent carryover contamination. UNG-treatment removes all dU-containing amplicons carried over from previous reactions.

IMPORTANT: UNG is not included in the 5x HOT FIREPol® Probe Universal qPCR Mix on must be purchased separately.

$MgCl_2$ concentration in the final reaction mix is 3 mM and further optimization is generally not necessary.

Reference dye based on ROX is included in the mix. Technology it is suitable for all qPCR platforms that require ROX for data normalization but does not interfere on platforms that do not need normalization. Adjustment of reference dye concentration is not necessary.

For multiplex application: if ROX dye is used as one of the fluorophores, reference dye might interfere with the signal – version without ROX is available upon request.

100% DMSO is included in the kit in separate vial. 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 Table 1. Volumes are given per reaction depending on final volume of reaction mix. Highest DMSO concentration recommended is 10% which should be used for all templates with GC content over 70%.

Table 1: Addition of 100% DMSO

FINAL CONC. OF DMSO	10 µL/RXN	20 µL/RXN
2,5 %	0.25 µl	0.5 µl
5%	0.5 µl	1 µl
7,5%	0.75 µl	1.5 µl
10%	1 µl	2 µl

Protocol

1. Thaw 5x HOT FIREPol® Probe Universal qPCR Mix, template (gDNA or cDNA), primer and probe solutions and PCR grade water at room temperature. Gently vortex and briefly centrifuge all solutions. Mix the 5x HOT FIREPol® Probe Universal qPCR Mix gently by swirling the bottle.
2. To minimize the possibility of pipetting errors prepare a reaction master mix (Table 2) for the number of reactions by adding PCR Grade water, primers and probe. This can be done at room temperature. **IMPORTANT:** Include 10% extra volume to compensate for the volume loss that occurs during pipetting

Table 2: Reaction master mix

COMPONENT	10 µL/RXN	20 µL/RXN	FINAL CONC.
5x HOT FIREPol® Probe Universal qPCR Mix	2.0	4.0	**1x**
Primer Forward (10 pmol/µl)	0.2-0.4 µl	0.4-0.8 µl	**200-400 nM**
Primer Reverse (10 pmol/µl)	0.2-0.4 µl	0.4-0.8 µl	**200-400 nM**
Probe solution	x µl	x µl	**100-250 nM**
100% DMSO[1]	variable	variable	**2,5%-10%**
UNG (Uracil-N-glycosylase)[2]	x µl	x µl	**x U/µl**
Template DNA[3]	0.5 -2.5 µl	1-5 µl	**0.001-2 ng/µl**
PCR Grade water	Up to 10 µl	Up to 20 µl	
Total volume per reaction	**10 µl**	**20 µl**	

[1] DMSO is recommended as a PCR additive for templates with high GC content. While testing it is recommended to include one sample with additional 2.5 % DMSO to test if it improves the results (follow Table 1 for instructions)

[2] Adding UNG is optional! UNG is not included in the 5x HOT FIREPol® Probe Universal qPCR Mix on must be purchased separately. Please add UNG according to manufacturer's specification.

[3] add on step 4

3. Mix the reaction master mix and aliquot it into individual qPCR tubes or the wells of qPCR plate.
4. Add template DNA into individual PCR tubes or the wells of qPCR plate.
5. Gently vortex the samples and spin down to collect drops.
6. Program the qPCR platform (table 3).

   IMPORTANT! Data acquisition should be performed during the annealing/extension step.
7. Place qPCR plate or tubes in the qPCR platform and start the program.

Table 3: Recommended qPCR cycles:			
CYCLE STEP	TEMP.	TIME	CYCLES
OPTIONAL! UNG treatment[1]	50°C	2 min	1
Initial activation[2]	**95°C**	**10 min**	1
Denaturation	95°C	15-20 s	40
Annealing/Elongation	60°C	60 s	

[1] ***OPTIONAL!*** *Add UNG treatment step ONLY if UNG enzyme is added in the reaction mix for carryover contamination removal*

[2] *To activate the polymerase, include an incubation step at* ***95°C for 10 minutes*** *at the beginning of the qPCR 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.