



EuroClone[®]
serving science through innovation

USER MANUAL
FluoCycle II™ for Probe
with Amplification Control
Ref.ERD001250

250 x 50 µl reactions
For in Vitro Diagnostic Use

Store at 2-8°C

1. Intended use

FluoCycle™ II Master Mix for probe provides a PCR mix that may be used with any appropriately designed primer and probe to detect any DNA or cDNA sequence. FluoCycle™ II Master Mix can be used for subsequent in vitro diagnostic purposes.

2. Kit Content

FluoCycle™ II Master Mix for probe is a 2X amplification mix for Real Time PCR protocols. In the kit is included an “*Amplification Control*” (β-globin) to verify the efficiency of your real time PCR protocols.

The real-time PCR probe-based technology uses a fluorogenic probe to enable the detection of a specific amplification product as it accumulates during PCR. This kit includes enough reagents to perform 250 reactions x 50 µl.

FluoCycle™ II Master Mix for probe contains a Hot Start Taq DNA polymerase (modified antibody technology) that can be activated by an initial step of 5 minutes at 95°C.



Reagents	Format	Formulation
FluoCycle™ II Master Mix	1.25 ml x 5 vials (250 reactions x 50 µl)	100mM KCl, 20mM Tris HCl pH 8.3, 0.02% Tween-20, 0.8mM of each dNTPs (dATP, dCTP, dGTP, dTTP), 200 units/ml Taq DNA polymerase, 8 mM MgCl ₂ , stabilizers.
Oligo Mix* for Amplification Control (Amber tube)	400 µl x 1 vial (20 reactions x 50 µl)	Primers and fluorogenic probe that bound covalently as a fluorophore at the 5'-end the HEX. The probe has at their 3'-ends a quencher called BHQ-1.
Amplification Control	200 µl x 1 vial	β-globin

***store the tube away from light**

3. Material required but not supplied

- ❖ Micropipettes
- ❖ Plugged tips
- ❖ Optical plates/tubes
- ❖ Thermalcycler with real-time PCR detection system
- ❖ Vortex
- ❖ Microcentrifuge
- ❖ Sterile bi-distilled water
- ❖ Forward and reverse primers for DNA target

4. Protocol for Master Mix Preparation (to use for DNA Target Amplification)

EuroClone recommends to prepare a PCR mix considering $n^{\circ} + 1$ samples. Add all reagents in a sterile tube and transfer the right volume of the resulting solution in the final reaction plate or tubes. Add the appropriate volume of DNA template. The reagents of PCR mix have to be mixed under the following ratio (see table 1 below):



Table 1: reaction set up

Reaction Component	Volume μ l	Final conc. – total quantity
FluoCycle™ II Master Mix for probe 2X	25	1X
Primers Forward	variable	50-900 nM
Primers Reverse	variable	50-900 nM
Probe	variable	50-300 nM
DNA template	variable	100 ng
Sterile Water	To reach 50 μ l	NA

5. Protocol for Master Mix Preparation (to amplify the Control of Amplification)

Inside the kit (ref. EDI001250 FluoCycle™ II Master Mix for Probe) is included an amplification control (β -globin) that can be used to verify the efficiency of amplification reaction if the technician consider useful to verify the performance of Real time PCR set. For one reaction the technician has to mix the reagents under the following ratio (see table 2) :

Table 2: reaction set up

Reaction Component	Volume μ l
FluoCycle™ II Master Mix for probe 2X	20
Oligo Mix for Amplification Control	20
Amplification Control (β -globin)	10
Final Volume	50 μ l

Transfer the tubes/plate inside the Real Time PCR Platform and runs the following program of amplification:

Thermal Profile

Time	Temperature	Cycles
5 min	95°C	1
15 sec	95°C	45
60 sec	60°C	Fluorescent Acquisition



The fluorescence signal registered by the instrument detects the presence of the DNA, in particular the fluorescence detected in the HEX channel reveals the Control of Amplification while the fluorescence detected in the other channel (FAM, Texas Red, Joe etc..) reveals the amplification of the Target DNA.

The Ct is defined as the cycle at which the amplification curve crosses the threshold line. The threshold line is calculated by the mean of fluorescence background multiply by three times standard deviation during the initial cycles of PCR, prior to significant accumulation of the target amplicon. During these early PCR cycles, the background signal is used to determine the “baseline fluorescence”.

The expected Ct of Amplification Control is between 25-27 threshold cycles.

6. Storage and Stability

All reagents should be stored at **2-8°C**. All reagents can be used until the expiration date printed on the labels.

7. Quality Control

FluoCycle™ II Master Mix for probe is free of contaminating DNAase and RNAase. Functionally the master Mix is tested to demonstrate resolution over three orders of human genomic DNA to detect a single copy gene, β -globin.

8. Comments

A. Primers and probes should be designed within a region including a G/C content ranging from 30% to 80%. Regions with G/C content higher than 80% might be not completely denatured during thermal cycling, causing lower amplification yield. G/C-rich sequences are also prone to non-specific interactions decreasing the amplification efficiency and producing non-specific signal in SYBR® Green dye assays. For the same reason, primers containing sequences of four or more G/C bases should be avoided.

B. To avoid non-specific annealing, primers should not contain more than two C/G repetitions at the last five positions of their 3' end. Maintaining the amplified fragment shorter than 150bp while satisfying this requirement might be difficult in case of highly G/C content. In general try to design primers with the lowest content of G/C repetitions at the 3' end.

C. The primers concentration should be optimised through an initial range from 50nM to 900nM. Because the individual efficiency of the forward and reverse primer may vary, their respective concentrations must vary to compensate. Therefore all permutations of a selected number of primer concentrations must be tested. For instance, there are nine possibilities of how forward and reverse primer concentrations could be combined for the following individual concentrations: 50nM, 300nM, 900nM:



Forward/Reverse: 50/50, 50/300, 50/900, 300/50, 300/300, 300/900, 900/50, 900/300, 900/900.

D. The probe concentration should be optimised through an initial range from of 50nM – 250nM. Three probe concentrations (50nM, 100nM, 250nM) should be combined with the nine forward and reverse primer concentrations above yielding 27 reactions. You will have your initial set of raw data so quantify each reaction. After quantitation analysis, the curve with the lowest Ct value and the highest amplification should be chosen for further experiments.

9. Ordering Information

Cat n°	Description	Format
ERD001250	FluoCycle II Master Mix for probe with Amp Ctrl	250 reactions X 50 µl
ERD002250	FluoCycle II Master Mix SYBR with Amp Ctrl	250 reactions X 50 µl
ERD001250R	FluoCycle II Master Mix for probe (ROX) with Amp Ctrl	250 reactions X 50 µl
ERD002250R	FluoCycle II Master Mix SYBR (ROX) with Amp Ctrl	250 reactions X 50 µl