Version: 4/2022

SHORT INSTRUCTION

FastQ® cff control

Thermocycler: Bio-Rad CFX

PCR PROGRAM

Step	Time	Temperature [°C]	Ramp Rate [°C/s]	Cycles
Initial Activation	10 min	95°C	2,5 °C/s	1
Denaturation	10 s	95°C	2,5°C/s	10
Annealing + Extension	1 min	60°C	2,2 °C/s	
	15 s	72°C	-	
Denaturation	10 s	95°C	2,5°C/s	35
Annealing + Extension	1 min	60°C	2,2 °C/s	
	15 s + plate read	72°C	-	

FLUOROPHORES

Specifity	Fetal marker	Internal Amplification Control (IAC)
Fluorophores	FAM	Texas Red

WORKFLOW

1. Step: Preparation	
	Thaw the 475 µl Q Mastermix fetal and 200 µl Q Primermix cff control vial at room temperature.
	Vortex the two tubes gently.

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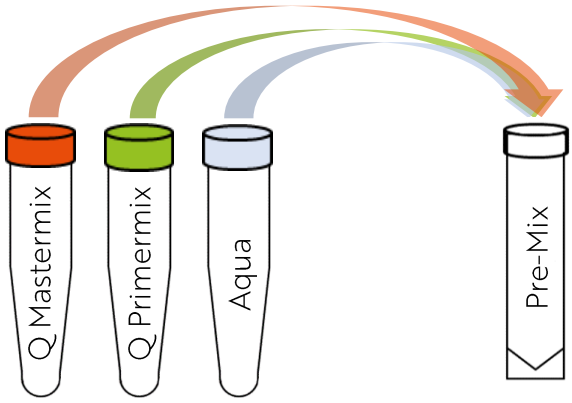
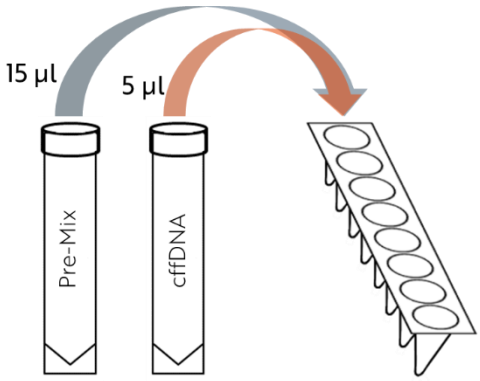
2. Step: Test setup

Please test the isolated *cell-free fetal DNA* (cffDNA) as triplicates with the following test-setup.

Prepare the Pre-Mix according to the number of samples and calculate an extra volume of 10% to account for pipetting loss. Pipette 15 µl from the pre-mix into each well and add 5 µl specimen cffDNA.

	Approach for single testing	Aproach Pre-Mix Triplicate: n (tests) = 3 *n (samples)
Q Mastermix fetal	10 µl	10 µl*n (tests) * 1,1 (10% extra)
Q Primermix cff control	4 µl	4 µl*n (tests) * 1,1 (10% extra)
A. dest	1 µl	1 µl*n (tests) * 1,1 (10% extra)
Volume	**15 µl**	





3. Step: Preparation

Close the reaction tubes and centrifuge briefly.

Place plate/strip into RT-cycler select "All Channels" and start the run.

When the cycler is under remote control of the Bio-Rad software, use the provided template.

4. Step: Evaluation

All signals $C_q \leq 30$ are in the correct-positive range.
For the detailed evaluation, please refer to the evaluation table in the instruction for use.

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