

SHORT INSTRUCTION

FastQ[®] RHD fetal

Thermocycler: Bio-Rad CFX



PCR PROGRAM

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

FLUOROPHORE

Specificity	Exon 5	Exon 7	Exon 10	Internal Amplification Control (IAC)
Fluorophore	FAM	VIC	Texas Red	Cyanin 5

WORKFLOW

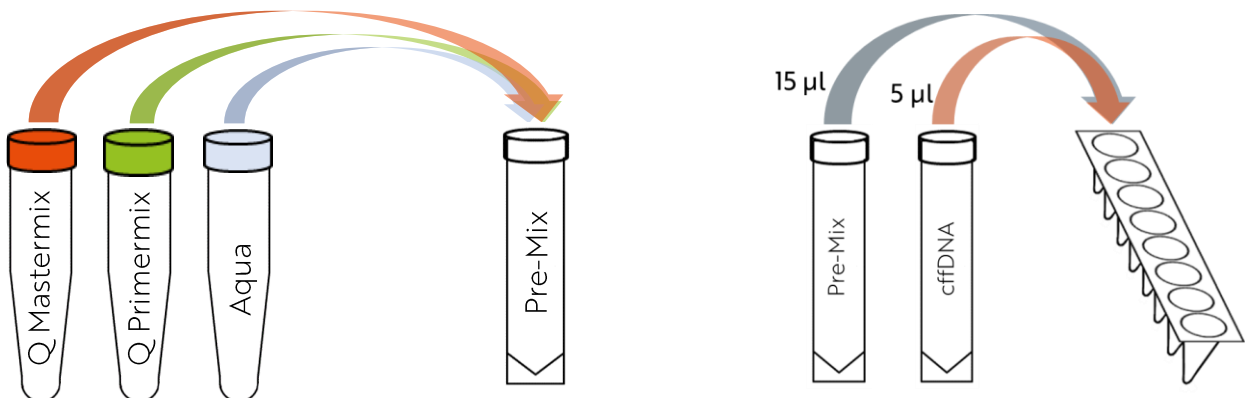
1. Step: Preparation	
	Thaw the 475 µl Q Mastermix fetal and 380 µl Q Primermix RHD fetal vial at room temperature.
	Vortex the two tubes gently.

2. Step: Test setup

Please test the isolated *cell-free fetal DNA* (cffDNA) as triplicate 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 (see table). Pipette 15 μ l from the pre-mix into each well and add 5 μ l specimen cffDNA.

No. of triplicate tests (n)	Q Mastermix fetal [μ l]	Q Primermix RHD fetal [μ L]	Aqua dest. [μ l]	Pre-Mix Volume [μ l]
1	40	16	4	60
2	70	28	7	105
3	100	40	10	150
4	130	52	13	195
5	160	64	16	240
6	190	76	19	285
7	220	88	22	330
8	250	100	25	375
9	280	112	28	420
10	310	124	31	465
15	470	188	47	705
20	630	252	63	945
25	780	312	78	1170
30	930	372	93	1395
32 (96 plate)	990	396	99	1485



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 following evaluation table.

Cy5 (IAC)	VIC (Ex 7)	Texas Red (Ex 10)	FAM (Ex 5)	Interpretation
Positive	At least 4 out of 9 replicates positive			● Positive.
Positive	1-3 out of 9 replicates positive			● The sample must be repeated.
Positive	Very early C _q -values compared to other results			● Not assessable, possibly mother with RHD variant-normal or weakly expressed D antigen or silent variant of the RHD gene*. The fetal RHD genotype cannot be determined.
Positive	Negative	2-3 replicates positive	Negative	● Partial rhesus D genotype. Mother* or child carry RHD-CE-D hybrid genes such as RHD-CE (2-9)-D, RHD-CE (3-9)-D, RHD-CE (3-7)-D, RHD-CE (4-7)-D. Section of RHD is replaced by the corresponding section of RHCE. In the case of RHD-CE-D hybrid genes, only exon 10 is positive.
Positive	2-3 replicates positive	2-3 replicates positive	Negative	● It may be a PSI-positive sample. In this case, further testing is recommended*.
Positive	Negative	Negative	Negative	● Negative: No specific exon was detected.
Negative	Negative	Negative	Negative	● Invalid: Invalid result due to real-time PCR inhibition, reagent failure or unsuitable test material.

* It is recommended to confirm the mother's rhesus D genotype (BAG Diagnostics ERY Q® RH, REF 728405; ERY Q® Weak D, REF 728401; ERY Q® Partial D, REF 728403).