

SHORT INSTRUCTION

FastQ[®] RHD fetal plus

Thermocycler:

Applied Biosystems QuantStudio™ 6 Flex Real-Time PCR System 96-Well Fast
oder QuantStudio™ 5 Dx System

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	2.2	
Denaturation	10 s	95	2.5	35
Annealing + Extension	1 min	60	2.2	
	15 s + plate read	72	2.2	
Cooling	2 min	37	2.2	1

FLUOROPHORES

Specificity	Exon 5	Exon 7	Exon 10	Fetal DNA- Control	Internal Amplification Control (IAC)
Fluorophores	FAM	VIC	Texas Red* (ROX)	TAMRA	Cyanin 5

* For RED Texas Red as well as ROX can be used.

WORKFLOW

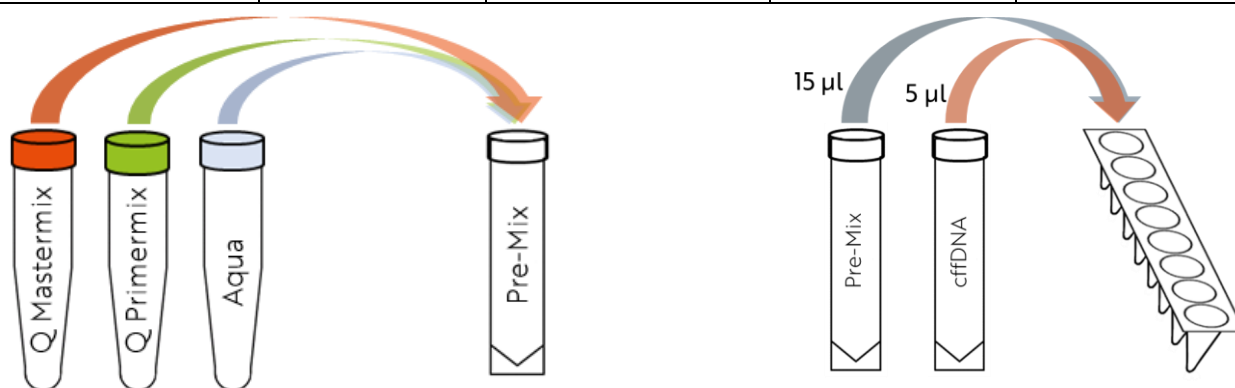
1. Step: Preparation	
	Thaw the 475 µl Q Mastermix fetal and 380 µl Q Primermix RHD fetal plus vial at room temperature.
	Vortex the two tubes gently.

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 (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 and start the run..

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.

CYCLER SETTINGS**1. Step: „Experiment properties“**

When working with Applied Biosystems QuantStudio™ 6 Flex Real-Time PCR System 96-Well Fast or a QuantStudio™ 5 Dx System, make the following basic settings:

Experiment properties*	QuantStudio™ 6 Flex Real-Time PCR-System 96-Well Fast	QuantStudio™ 5 Dx Real-Time PCR-System, 96-Well, 0.2 ml
Instrument	Quantstudio™ 6 Flex System	Quantstudio™ 5 Dx
Block	Fast 96-Well (0.1ml)	96-Well 0.2-mL
Experiment Type	Comparative Ct	Comparative Ct
Chemistry	TaqMan® Reagents	TaqMan® Reagents
Run Mode	Standard	Standard
Reaction Volume	20 µL	20 µL
Heated Cover Temperature	105.0°C	105.0°C
Passive Reference	None	None

2. Step: „Define“ / „Plate“ Selection of reporters and quenchers

On this RT-cyclers, the reporters and quenchers can be selected. A reporter can only be selected if the dye has been calibrated beforehand. The reporters must be selected on a kit-specific basis and depend on the probes to be detected in the respective kit. The reporter name and colour is selected by each laboratory during calibration. For the quencher, NFQ-MGB should be selected for all kits.

Cy5 (IAC)	TAMRA (Fetal marker)	VIC (Ex 7)	Texas Red (Ex 10)	FAM (Ex 5)		Interpretation	
Positive	At least 2 out of 3 replicates positive	At least 4 out of 9 replicates positive				●	Positive.
Positive	1 out of 3 replicates positive	1-3 out of 9 replicates positive				●	Negative, repetition is recommended.
Positive	2-3 replicates positive	Very early Cq-values compared to other results				●	Not assessable, maternal RHD variant possible - normal or weakly expressed D antigen or silent variant of the RHD gene*. The fetal RHD genotype cannot be determined.
Positive	2-3 replicates 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	2-3 replicates positive	Negative		●	It may be a PSI-positive sample. In this case, further testing is recommended*.
Positive	Negative	Negative	Negative	Negative		●	Negative: No specific exon was detected. Fetus RHD negative and the fetal marker not present or the sample does not contain detectable or sufficient copy numbers (LOD) of the cffDNA.
Positive	2-3 replicates positive	Negative	Negative	Negative		●	Negative: Sufficient cffDNA was entered in the test.
Negative	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).