

Free DNA Fetal Kit[®] RhD

**Earliest Detection Combined With The Most Specific Method
by Real-Time PCR!**

- Earliest detection of cell-free fetal DNA from plasma of RhD-negative pregnant women
- No impact on pregnancy
- High specificity due to real-time PCR test method
- Standardized and reliable
- Avoids unnecessary prophylaxis treatments
- Primers for *RHD* Exon 5, 7 and 10



Dx Real-Time System and Free DNA Fetal Kit[®] RhD

The Complete Solution for Safe Transfusion

BIO-RAD



Free DNA Fetal Kit® RhD

Anti-D Immunization

In the absence of prophylaxis, anti-D immunization is a significant cause of hemolytic disease of the fetus and newborn (HDFN). This severe condition is most often caused by the destruction of fetal RhD-positive red blood cells, induced by anti-D antibodies present in the blood of an immunized RhD-negative mother after crossing the placenta.

Anti-D antibody production can be prevented by immunoprophylaxis, consisting of passive administration of anti-D immunoglobulins to RhD-negative mothers.

Determination of the fetal RHD genotype is essential:

- for establishing whether a non anti-D immunized RhD-negative pregnant woman requires appropriate immunoprophylaxis,
- for the management of pregnancy at risk: RhD-negative pregnant women with anti-D, to assess whether the fetus is at risk of HDFN.

Test Principle

Prenatal determination of the *RHD* genotype became a reality following two major findings:

- cloning of the *RH* (*D* and *CE*) genes^{1,2},
- and elucidation of the molecular basis of RhD-pos/RhD-neg polymorphism³.

These studies have shown that the *RH* locus of RhD-positive individuals is composed of two homologous genes *RHD* and *RHCE*, encoding for the RhD and RhCcEe proteins, respectively, and that the *RHD* gene is deleted in RhD-negative individuals of Caucasian origin.

Based on these findings, a first generation of invasive genotyping methods was established by PCR amplification of exon 10 (of the *RHD* gene), using amniotic fluid or chorionic villus samples in early pregnancy^{4,5}. With increasing knowledge of the molecular basis of a large number of *RH* variants, the discovery of the *RHD* pseudogene (*RHD* ψ) frequently found in black individuals⁶, and the presence of cell-free fetal DNA in the maternal plasma⁷, a second generation of non-invasive PCR methods based on the use of several primer pairs for the simultaneous detection of different *RHD* gene regions (exons or introns) in maternal plasma was designed in many laboratories, mainly in Europe. The majority of *RH* variants could be detected (but not identified) using this new concept, thus reducing the risk of potentially harmful false-negative results.

The concentrations of fetal DNA in the plasma of pregnant women increase with gestational age. In fact, fetal DNA concentrations (fetal genome per ml of plasma) increase

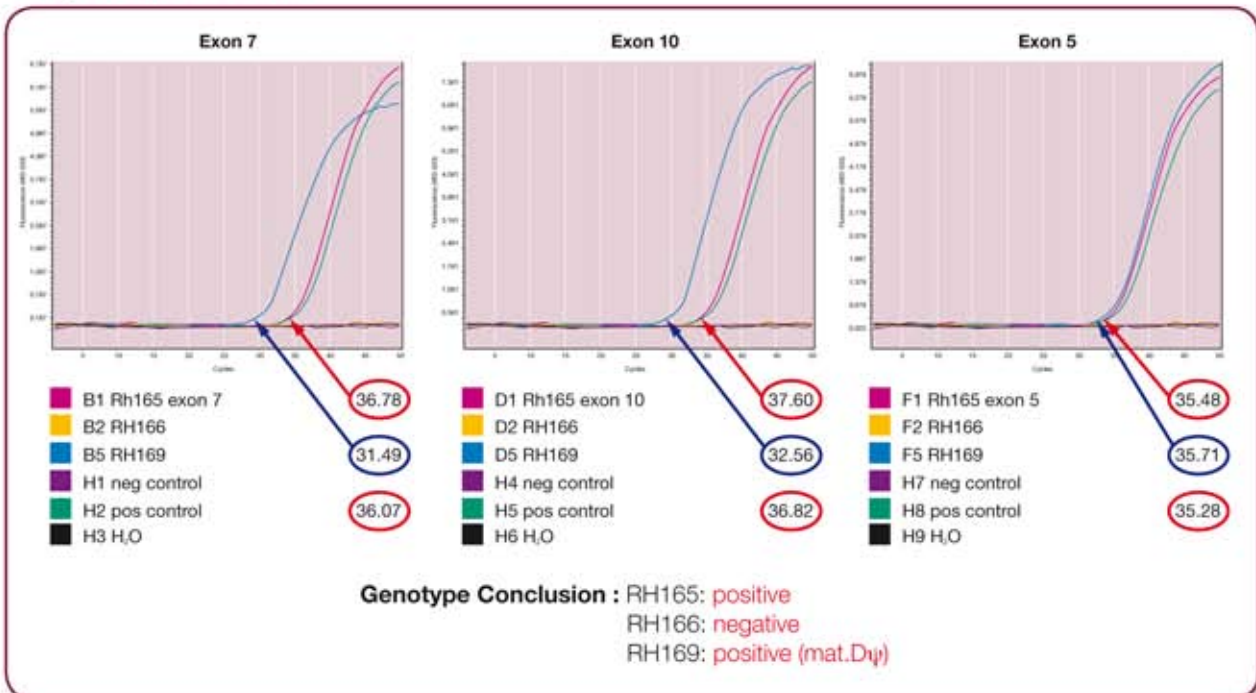
from a few copies in the first trimester of pregnancy up to several hundred copies in the third trimester.

The first generation of the *RHD* genotyping kit was based on the amplification of two *RHD* specific regions in exon 10 (highly conserved) and exon 7 (absent in some variants) by real-time PCR^{8,9}. The second generation now developed in the present kit includes amplification of a third specific region located in exon 5 that could detect exon 5 from the *RHD* gene, but not from the *RHD* ψ pseudogene¹⁰. Accordingly, genotyping exon 5D (not *D* ψ) will allow genotyping of fetuses in mothers carrying the *RHD* ψ pseudogene. Thus, in addition to the fetal *RHD* status (positive or negative) the presence of the main known *RHD* variants will be detected.

Consequently, the *RHD* gene identified by PCR in the plasma of pregnant RhD-negative women (Oxford University patent) is of fetal origin. Total DNA is extracted from maternal plasma for each fetal *RHD* genotype analysis. The presence of a fetal *RHD* gene in the plasma DNA is detected by real-time PCR amplifications of three different segments of the *RHD* gene (exons 5, 7 and 10), in order to detect the largest number of *RHD* variants. Each amplicon is detected with specific hydrolysis probes.

[1] Chérif-Zahar B et al., Proc Natl Acad Sci USA 1990, 87:6243-7; [2] Le van Kim et al., Proc Natl Acad Sci USA 1992, 89:10925-9; [3] Colin et al., Blood 1991, 78:2747-52; [4] Bennett et al., N Engl J Med 1993, 329:607-10; [5] Lo et al., N Engl J Med 1993, 341:1147-8; [6] Singleton et al., Blood 2000, 95:12-5; [7] Lo et al., N Engl J Med 1998, 339:1734-8; [8] Rouillac et al., Mol Diagn 2004, 8:23-31; [9] Rouillac-Le Sciellour et al., Transfus Clin Biol 2007, 14:572-7; [10] Finning et al., Transfusion 2002, 42:1079-85.

Amplification Curve



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Test Performance

A study was conducted on 300 samples taken from the plasma of pregnant women of RhD-negative phenotype (C. Rouillac-Le Scieillour, V. Sérazin, Y. Brossard, O. Oudin, C. Le Van Kim, Y. Colin, Y. Guidicelli, M. Menu, J.P. Cartron Noninvasive fetal *RHD* genotyping from maternal plasma. Use of a new developed Free DNA Fetal RhD. *Trans Clin Biol* 2007; 14:572-7) (Read the full article). The results were compared with the phenotype RhD of the newborn child, as well as in the technique initially developed by C. Rouillac-Le Scieillour and al. (C. Rouillac-Le Scieillour, P. Puillandre, R. Gilot, C. Baulard, S. Métral, C. Le Van Kim, J-P. Cartron, Y. Colin, Y. Brossard, Large-scale pre-diagnosis study of fetal RhD Genotyping by PCR on Plasma DNA from RhD-Negative pregnant Women, *Mol Diagn* 2004; 8:23-31).

- 100% of correlations were obtained between the two methods.
- No false-negative result (defined as the absence of amplification of *RHD* exons) was found in children with a RhD-positive phenotype. The genotyping in the plasma of all the mothers whose children were phenotypically RhD-positive revealed the presence of the fetal *RHD* gene. At present, however, a false-negative result cannot be excluded in the absence of a universal fetal DNA marker.

Limits

This test is validated exclusively for the analysis of human plasma collected with EDTA or ACD anticoagulants. Heparin inhibits PCR and must not be used with this method. It is advisable to perform this test on samples taken from 12 weeks of amenorrhea (sufficient level of circulating fetal DNA).

A **negative** fetal *RHD* genotype from an initial maternal blood sample taken before 18 weeks of amenorrhea must be considered as probable, and has to be confirmed on a second maternal blood sample collected at least 2 weeks later in order to rule out a false-negative result.

A **positive** fetal *RHD* genotype from an initial maternal blood sample can be considered as acquired.

A **false-positive** result is possible from cross-contamination, or if the *RHD* fetal haplotype is a silent variant.

Kit contents

- **RHD positive (+) Control:** 6 x 1,000 µl (red top)
- **RHD negative (-) Control:** 6 x 1,000 µl (green top)
- **[100X] Maize DNA Control (not ready to use):** 3 x 14 µl (yellow cap insert)
- **Maize exon IVR2 primers sense/antisense + probe:** 3 x 38 µl (green cap insert)
- **RHD exon 5 primers sense/antisense + probe:** 3 x 38 µl (purple cap insert)
- **RHD exon 7 primers sense/antisense + probe:** 3 x 38 µl (white cap insert)
- **RHD exon 10 primers sense/antisense + probe:** 3 x 38 µl (red cap insert)

Additional Equipment and Reagents Required

The following equipment and reagents are not included but are required for performing the assay:

- **Equipment and associated accessories:**
 - QIAamp® MinElute® Virus Vacuum Kit 50 columns, QIAGEN ref. 57714,
 - or
 - QIAamp DSP Virus Kit (IVD CE) 50 columns, QIAGEN ref. 60704
 - Bio-Rad Dx Real-Time System (IVD CE) ref. 94000
- **Reagent (reactive) real-time PCR:**
 - For LightCycler®: LightCycler® Taqman® Master Roche®, ref. 04 535 286 001 (50 reactions), or ref. 04 735 536 001 (480 reactions).
 - Further instruments (ABI, MX): Faststart Taqman® ProbeMaster Roche®, ref. 04673409 (100 reactions).
- **Consumables for real-time PCR instruments,** with system glass capillaries (20 µl), or with multi-well plate.
- **Nuclease-DNA free labware:** pipettes and specific tips with filters for PCR.
- **Molecular biology grade water.**

Ordering Information

Catalog No.	Description	
060001	Free DNA Fetal Kit® RhD	87 tests

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