FetoGnost® Kit RHD

Kit Version 1.1

For *in vitro* diagnostic use

FetoGnost® Kit RHD	Kit Version 1.1		
Order no.	**Reactions**	**Exon 5, 7, 10**	**Internal positive control**
HUFG100	100	VIC+FAM+NED channel	Cy5 channel
HUFG500	500	VIC+FAM+NED channel	Cy5 channel

Kit contents:

- Detection assay for the human *RHD* gene, exons 5, 7 and 10 and for the IPC (internal positive control)
- Target for DNA internal positive control (DNA IPC, control of PCR amplification and/or DNA extraction)
- DNA reaction mix for real-time PCR (contains a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs, ROX™ dye (passive reference) and buffer components – additives optimized to handle PCR inhibitors)
- DNA Positive control for exons 5, 7 and 10

Background: Rhesus factor D (RhD) or RhD antigen is the most common of the five main Rhesus antigens (C, c, D, E, and e) out of a variety of antigens on the surface of red blood cells. The dominantly inherited *RHD* gene determines whether a person is RhD-positive or -negative. Most of the population is RhD-positive. In a RhD-negative status, a complete deletion of the *RHD* gene is usually present. Less commonly, minor genetic alterations (e.g., point mutations, insertions, deletions, and gene rearrangements) result in non-functional or weak RhD antigen.
Prediction of the fetal RhD status is significant for the prevention of fetal haemolytic disease, where a RhD-negative mother becomes sensitized to an RhD-positive fetus causing a maternal immune response to produce IgG anti-D antibodies, which damage the fetus in a subsequent pregnancy.

Intended purpose: FetoGnost® Kit RHD is a non-automated CE-certified IVD real-time PCR test for the qualitative detection of fetal *RHD* DNA from extracted human maternal plasma of non-immunized RhD-negative pregnant women (non-invasive prenatal determination of fetal *RHD* status, NIPT-RHD). The test detects exons 5, 7 and 10 of the *RHD* gene.
This test is suitable for women of all ages with gestational age ≥ 11+0 with singleton or multiple pregnancies. It can be used both in a first pregnancy and in subsequent pregnancies. It allows targeted anti-D prophylaxis in RhD-negative pregnant women without anti-D alloimmunization.

Contraindications:

- Pregnant women with anti-D alloimmunization. Thus, the immunization status of the pregnant woman should be known before starting the test.
- The test is not suitable for samples taken before gestational age 11+0. The limited performance of the test prior to gestational age ≥ 11+0 must be indicated on the report.
- The test is not intended for Rhesus D determination of transfusion recipients and blood donors.

The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.

Three probe-specific amplification-curves in VIC, FAM and NED channel indicate the amplification of exon 5, exon 7 and exon 10 of the *RHD* gene, respectively. In addition, an internal positive control (IPC) with detection in Cy5 channel monitors the integrity of kit reagents, serves as a control for DNA extraction and excludes false-negative interpretation of results due to inhibition of real-time PCR. The target for the IPC is added during DNA extraction of maternal plasma samples.
The sensitive and robust multiplex test format for the detection of three exons of the *RHD* gene in triplicates minimizes false-negative results.

PCR-platforms: FetoGnost® Kit RHD has been validated with the ABI® 7500 Fast instrument and QuantStudio™ 7 Pro (Thermo Fisher Scientific) - fast cycle parameters are not supported by this test. It is, however, also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM, VIC, NED, ROX and Cy5 channels.

Sensitivity and specificity: The detection limit (LoD95%: number of copies, which are positively detected in 95% of cases) for exons 5, 7, and 10 detection is 13, 8, and 7 target copies/reaction, respectively. The test is 100% specific for human *RHD* gene exon 5, 7 and 10. The diagnostic sensitivity is 99.64% and the diagnostic specificity is 98.92%.

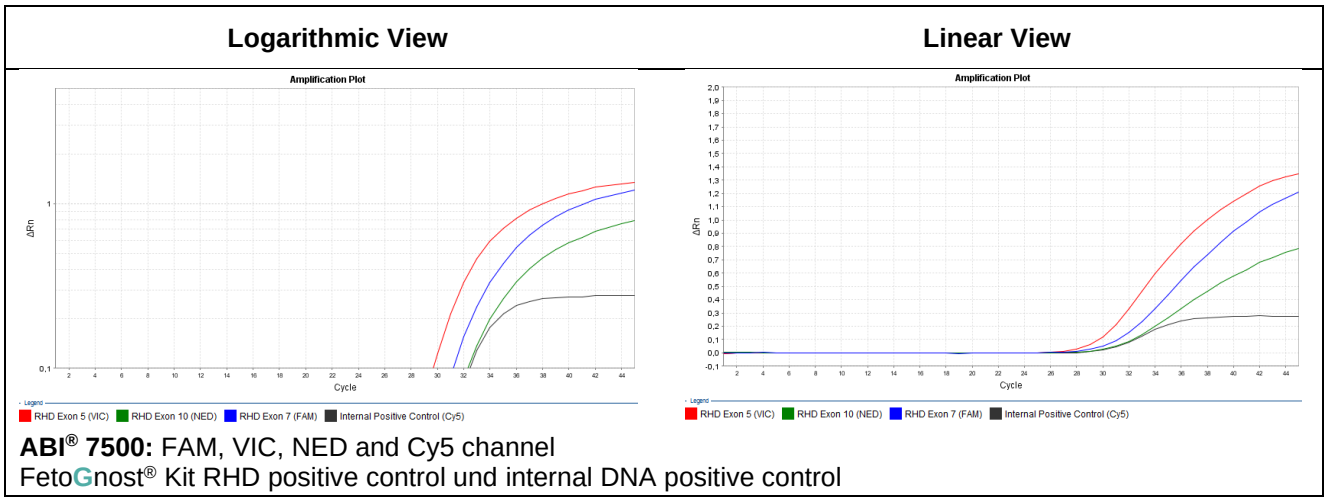

Figure 1 Performance of FetoGnost® Kit RHD

References:

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- Legler, T.J., Lührig, S., Korschineck, I. and Schwartz, D. 2021. Diagnostic performance of the noninvasive prenatal FetoGnost RhD assay for the prediction of the fetal RhD blood group status. Archives of Gynecology and Obstetrics. 2021 Apr 9 (doi:10.1007/s00404-021-06055-1. Epub ahead of print)
- Müller SP, Bartels I, Stein W, Emons G, Gutensohn K, Köhler M, Legler TJ. 2008. The determination of the fetal D status from maternal plasma for decision making on Rh prophylaxis is feasible. Transfusion. 48:2292-301.