Datasheet v3.0

Cell3™ Direct: Rhesus D Fetal Blood Group Genotyping Kit

The first direct from plasma non-invasive prenatal kit for fetal RhD genotyping

Highlights

Direct from maternal plasma

No cfDNA extraction required. Simple real-time qPCR protocol delivers accurate results direct from <0.25 ml of plasma in under 3 hours.

Reduced costs

Direct from plasma approach saves technician time and extraction costs associated with current home brew methods.

Flexible and validated kit

96 well formatting within a break-apart plate allows for between 4 and 13 samples per kit while the validated protocol ensures robust and accurate reporting of findings.

Multi-target assay approach

Amplification of multiple targets (RhD Exons 5, 7, 10) improves sensitivity as compared to single target assays due to intra assay concordance.

Introduction

In the absence of anti-D prophylaxis, anti-D immunization in Rhesus D (RhD) negative women carrying an RhD positive fetus is a significant cause of Hemolytic Disease of the Fetus and Newborn (HDFN). This occurs when maternal IgG anti-D antibody, produced as a result of prior exposure to Fetal RhD positive red cells present in the maternal circulation, cross the placenta and cause Fetal RhD positive red cell hemolysis resulting in anemia.

The anti-D antibody production and thus HFDN can be prevented by immuno-prophylaxis consisting of the administration of anti-D immunoglobulin to RhD negative mothers. Unfortunately this results in a significant percentage[1] (38%) of RhD negative mothers receiving the anti-D human blood products unnecessarily.

Genetic testing of cell free fetal DNA (cffDNA) present in maternal circulation during pregnancy, is changing the diagnostic care pathways in prenatal healthcare. Recent NICE recommendations[2] and publications[3] demonstrate the care pathway, diagnostic and health economic benefits of performing RhD fetal testing using Non-Invasive Prenatal Diagnosis (NIPD) as opposed to the current practice of providing all RhD negative pregnancies with a prophylactic treatment.

nonacus

ADVANCING NON-INVASIVE HEALTHCARE

NICE recommends test to identify fetal rhesus D status (to the UK, National Health Service)

"Maternity services should start offering pregnant women who are rhesus negative a test to identify the status of their fetus, under final guidance issued by the National Institute for Health and Care Excellence. Non-invasive prenatal testing for fetal rhesus D status is carried out during routine antenatal appointments and analyzes the baby's DNA found in its mother's blood. This test means that only women whose baby is rhesus D positive will be treated with anti-D immunoglobulin, saving the NHS more than £500,000 (€582,000; $624,000) a year spent treating 40,000 women who do not need anti-D".
BMJ 2016; 354: i3944

How it works

The Cell3 Direct RhD kit targets sequences specific for exons 5, 7 and 10 of the RhD gene and can distinguish between RhD positive, negative and RhD Psi genotypes in cffDNA and in a background of maternal cell free DNA (cfDNA). A control gene (CCR5) is also included to confirm adequate cfDNA quantity within the sample.

To allow for ambient temperature shipment of our products we have assessed the stability of our kit by leaving the relevant components at room temperature for up to 35 days. The results demonstrated that the assay is robust and performance was not affected over this time.

Complete kit convenience

The Cell3 Direct RhD kit contains pre-plated primers and probes in a flexible break-apart 96 well format allowing for 1 to 13 patients to be tested per run (based on 3 replicates per assay). The kit includes all components for testing direct from plasma or from extracted cfDNA using any common qPCR / Real-Time PCR System. Positive and negative controls are included in the kit for completeness.

Data quality

Technical sensitivity of the assay was demonstrated (Figure 1) on artificially prepared genomic DNA samples containing RhD negative DNA spiked in with 1 or 10% (equivalent to 3 or 30 genomic equivalents) RhD positive DNA. Samples containing only RhD positive or negative genomic DNA were also included as controls.

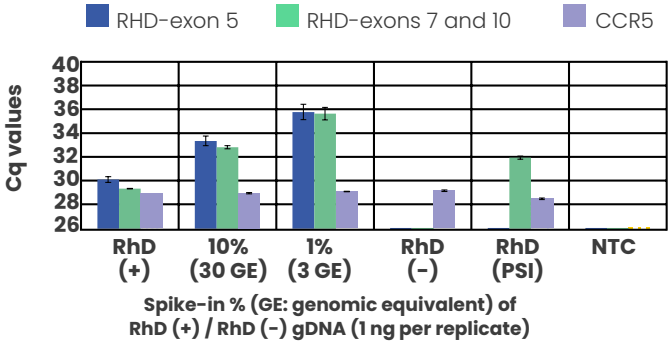

Figure 1: Assay sensitivity and specificity test with spike-in genomic DNA samples.

Direct from plasma protocol was compared with extracted cffDNA and comparable results were demonstrated using the same plasma sample (RhD positive fetus, 24 weeks' gestation) (Figure 2a and 2b).

Sensitivity and specificity of our kit were assessed on 100 plasma samples (24–26 weeks gestation). All samples were run using Cell3 Direct RhD kit according to the protocol. The samples were blinded and analyzed independently using parameters comparable to that used in a clinical setting.

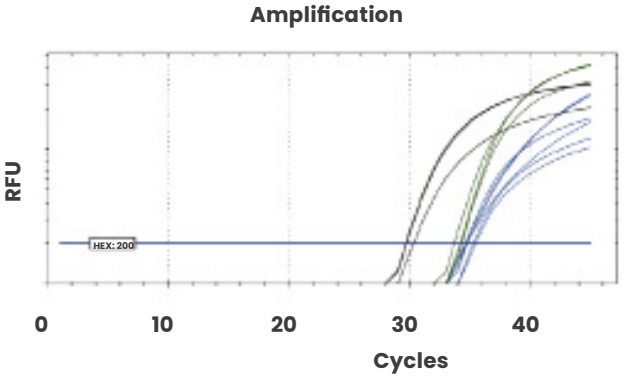

Figure 2a: Extracted cfDNA amplification.