Cell3™ Direct: Fetal Sex Determination Kit

The first direct from plasma, non-invasive prenatal test to determine Fetal Sex with sex-linked disease

Highlights

- **Direct from Maternal Plasma**
  No cfDNA extraction required. Simple Real-Time qPCR protocol delivers accurate results direct from <0.25ml 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 (SRY, TSPY and DAZ) improves sensitivity as compared to single target assays due to intra assay concordance.

Introduction

Knowledge of fetal gender is critical to the management of pregnancies at higher risk of sex-linked disease. Sex-linked disease is typically caused by a pathogenic mutation on the single male X chromosome and hence is frequently manifested in males with a strong family history.

The most common X-linked recessive diseases include Hemophilia and Duchenne Muscular Dystrophy (DMD). Whilst each disease is individually relatively rare, it has been estimated that in combination they occur in around 5 in 10,000 live births[1].

Although fetal sex can often be determined using an ultrasound scan of the fetus in the second or third trimester, a definitive prenatal diagnosis can currently only be made through invasive testing, using either chorionic villus sampling (CVS) at 11–14 weeks' gestation, or amniocentesis from 15 weeks' gestation. Because both these invasive techniques carry a small but significant risk of miscarriage[2] (0.5-1%), many women are reluctant to undergo invasive testing. However, there are substantial advantages to earlier diagnosis. Where future management might involve a decision to terminate the pregnancy, early termination carries fewer risks.

Utilizing circulating cell free fetal DNA (cffDNA), which can be detected in maternal plasma from early in gestation, and through the use of our proprietary Cell3™ amplification technology the Cell3™Direct Fetal Sex Determination kit accurately reports fetal sex, early in pregnancy, without the need for invasive procedures.

How it works

Cell3™Direct Fetal Sex Determination Kit targets specific sequences on Chromosome Y: SRY, TSPY and DAZ. Also included is a control gene (CCR5) to confirm there is an adequate amount of cell free DNA (cfDNA) within the sample. The sex of the fetus is determined by the presence of Y-specific sequences with intra-assay concordance for a male fetus and the absence of Y specific DNA sequences in the case of a female fetus.

Complete Kit Convenience

The Cell3™Direct Fetal Sex Determination Kit contains pre-plated primers and probes in a flexible break-apart 96 well format allowing from 1 to 13 patient tests per run (based on 3 replicates per assay). All components for direct from plasma or extracted cffDNA amplification using any common qPCR / Real-Time PCR System are included together with positive and negative controls.

Data Sheet: NIPD for Fetal Sex Determination

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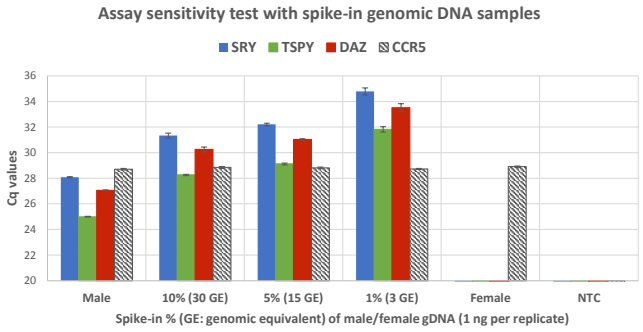
Data Quality

Technical sensitivity of our assay was demonstrated (figure 1) using male gDNA spiked into a female gDNA background at fixed percentages to reproduce realistic fetal fractions and confirm the sensitivity of the assay. Amplification of all targets and across all replicates was observed even at the lowest 1% spike-in.

Figure 1



Direct from plasma protocol was compared with extracted cfDNA and comparable results were demonstrated using the same plasma sample (male fetus, 10 weeks' gestation) (figure 2a and 2b). SRY (blue), DAZ (red), TSPY (green), CCR5 (black).

Figure 2a (extracted cfDNA amplification)

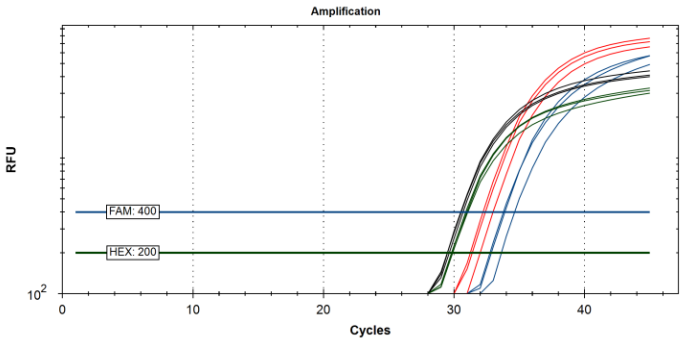
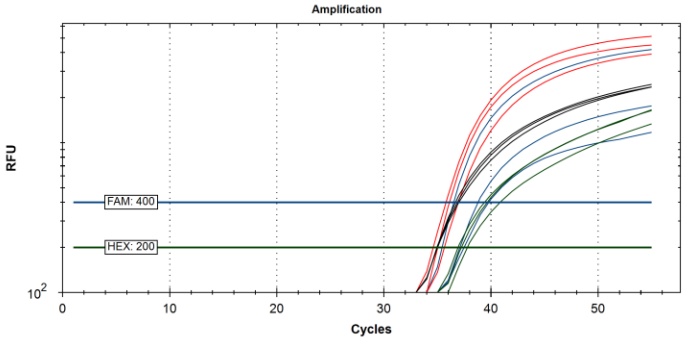


Figure 2b (Direct from plasma amplification)



Sensitivity and specificity were assessed using fifty plasma samples (10-27 weeks gestation, median 15). All samples were run using Cell3™Direct – Fetal Sex Determination Kit using x3 replicates for each assay. The samples were blinded and analyzed independently using parameters comparable to that used in a clinical setting. Results were concordant in 49/50 with 1 inconclusive result which once re-run using the same settings was also called correctly.

Summary

The Cell3™Direct Fetal Sex Determination Kit is the first commercially available direct from plasma non-invasive prenatal test for fetal sex determination. This kit sets a new benchmark in simplicity of setup with results generated within 3 hours from sample receipt. The protocol offers significantly reduced hands-on time due to no sample extraction requirement and flexible pre-plated assays. Total plasma quantity required is <0.25ml per sample enabling a smaller volume of blood to be drawn or retention of remaining sample for other tests to be undertaken if required.

Learn More

To learn more about the Cell3™Direct Fetal Sex Determination Assay and to download the protocols, MSDS, application notes, and white papers please visit our website: www.nonacus.com or email: info@nonacus.com

Ordering Information

Product	Catalogue No.
Cell3™Direct Fetal Sex Assay kit (up to 13 samples) – Low Profile QPCR Plates	C3101LP
Cell3™Direct Fetal Sex Assay kit (up to 13 samples) – High / Standard Profile QPCR Plates	C3102SP

If in doubt about which part number to order, please visit our website: www.nonacus.com or email: info@nonacus.com

References

1. Baird PA, Anderson TW, Newcombe HB, Lowry RB: Genetic disorders in children and young adults: a population study. Am J Hum Genet 1988, 42(5):677–693

2. Akolekar, R., Beta, J., Picciarelli, G., Ogilvie, C. and D'Antonio, F. (2015), Procedure related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis Ultrasound Obstet Gynecol, 45: 16–26 doi:10.1002/uog.14636

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