

Cell3™ Xtract: cell-free DNA Extraction Kit

Fast and flexible cell-free DNA extraction kit from plasma and other biological specimens

Highlights

- Fast and Convenient Protocol**
 Fast and simple protocol enables a 90 minute processing time with 45 minutes hands-on time, to extract cfDNA from a 1-10ml volume. Requires centrifugation steps only. No specialist equipment such as magnets or vacuum manifolds needed
- Flexible Kit**
 Flexible input volume of 1-10ml enables increased cfDNA recovery and use of the entire sample volume
- Supports Low Input DNA and Sensitive Applications**
 Cell3™ Xtract technology enables an elution volume of as low as 35µl, which helps avoid the need for DNA concentration steps and assists with low input or sensitive applications such as quantitative/real-time/digital PCR and Next Generation Sequencing
- Multi-Specimen Support**
 Extract cfDNA from plasma, cerebrospinal fluid (CSF), saliva, serum and amniotic fluid
- Accurate Quantitation of cfDNA**
 Accurate quantitation of extracted cfDNA is important for downstream applications, particularly Next Generation Sequencing where input amounts for assays can be critical. Many companies use carrier RNA to improve cfDNA extraction which can lead to false/incorrect cfDNA quantitation. The Cell3™ Xtract kit does not require carrier RNA and therefore enables accurate quantitation of cfDNA

Introduction

The utility of cell-free DNA (cfDNA) in both translational research and diagnostic settings has increased dramatically since its discovery. Areas of research being undertaken which involve cfDNA are diverse and include prenatal, oncology, transplantation and ageing. CfDNA is typically found in short fragment sizes of ~160bp and varying but low quantities of 1 to 100 ng/ml in plasma. It is therefore critical that sufficient cfDNA of good quality can be extracted for downstream applications. To address this need we have developed a simple and flexible cfDNA extraction protocol.

Quick and Convenient Workflow

Cell3™ Xtract has a very simple and flexible workflow which allows 1-10ml of biological sample to be processed within 90 minutes with around 45 minutes hands on time. The flexible input volume allows for increased recovery and concentration of cfDNA while avoiding the necessity for multiple 1ml extractions. No specialist equipment such as magnets or vacuum manifolds are required, just a microcentrifuge, a swinging bucket centrifuge capable of holding 50ml tubes, and a heat block or water bath (55°C).

Reduced Elution Volume

A flexible elution volume of ≥35µl avoids the need for DNA concentration steps, which can cause additional sample loss.

Validated Kit

To ensure that our kit performs to our exacting standards, we compared it to manual cfDNA extraction kits from 4 other companies, including the market leading company (companies Q, A, O, M). These kits covered both spin column and bead based methods. For kit performance evaluation, we used plasma from pregnant women carrying a male fetus.

We extracted cfDNA from 1ml of plasma following the manufacturer's recommended protocol and ensuring that we used the same patient sample for comparison between the Nonacus Cell3™ Xtract kit and other vendor kits.



Data Sheet: cell-free DNA Extraction Kit

Quantitative PCR data revealed that the Cell3™Xtract kit performed on par with the Company Q kit and outperformed the Company A kit. However, the use of carrier RNA in the Company Q kit resulted in a higher DNA concentration measurement (~50 fold) and the appearance of a high molecular weight peak in the fragment analysis electropherogram.

Comparison to spin column based kits.

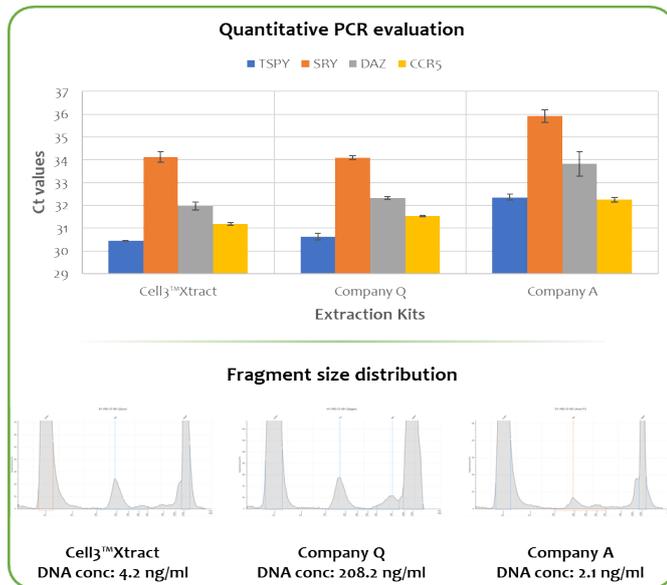


Figure 1. cfDNA was extracted from 1ml of plasma for each kit: the same sample from a male pregnancy at 14 weeks gestation was used. Quantitative PCR evaluation was conducted to detect and compare the extracted fetal cfDNA portion using the Cell3™Direct: Fetal Sex Determination kit (Nonacus), which includes chromosome Y specific targets (TSPY, SRY, DAZ) and a control target (CCR5). Fragment size distribution was measured using high sensitivity reagents on the TapeStation 2200 (Agilent). DNA concentration was determined using high sensitivity reagents on the Qubit 3.0 (Invitrogen).

Summary

When comparing its performance to other manual cfDNA extraction kits available on the market, we have found that the Cell3™Xtract kit delivers:

- a higher recovery of cfDNA (compared to Companies A and M)
- a cleaner sample by not requiring the use of carrier RNA (compared to Company Q)
- a faster workflow (compared to companies Q, A, M, O)
- a more flexible approach to cfDNA extraction (compared to company Q)
- a simple protocol which does not require the use of specialist equipment (companies Q, M, O) Therefore, we have developed a fast, flexible and effective cfDNA extraction kit which allows multiple input volumes, choice of biological input, reduced elution volume and does not require specialist equipment

In the comparison with bead based kits, the Cell3™Xtract kit performed better than the Company M kit (based on quantitative PCR data and DNA concentration measurements) and on par with the Company O kit.

Comparison to bead based kits.

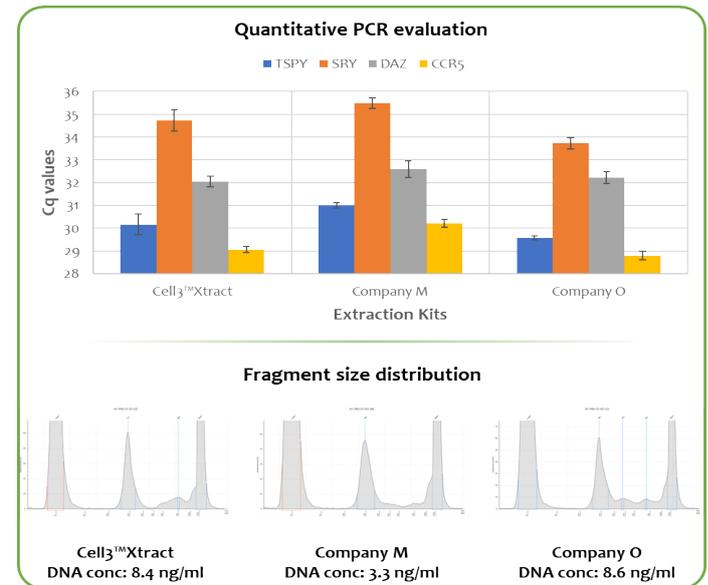


Figure 2. cfDNA was extracted from 1ml of plasma for each kit: the same sample from a male pregnancy at 10 weeks gestation was used. Quantitative PCR, fragment size analysis and DNA concentration measurements were conducted as explained in figure 1.

Ordering Information

Product	Catalogue No.
Cell3™ Xtract kit - 16 sample kit	C3016SK
Cell3™ Xtract kit - 48 sample kit	C3048LK

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

