

DNA extraction from low cellularity samples.

iGENatal™ Kit. IGEN Biotech.

- ❑ High yield, high purity, high quality from as few as 50,000 cells
- ❑ Fast and easy
- ❑ R&D supported by the Spanish government

About Igen Biotech

IGEN was launched in 2008 as a "bio-incubator", offering hosting management and administration services of laboratory equipment and services to clientele, primarily premiere research centres in Madrid, while at the same time investing in a diversified portfolio of biotechnology products and services. Thus, its core business is diversified, consisting not only of bridging R&D to market, but also providing management and laboratory services to the public. It has created a team that provides value to the company in both the scientific and management aspects of the technology transfer process. In 2010, the company put in place an effective marketing plan that has opened up new research collaborations that have the potential to bring cutting-edge research to market.

iGENatal™, the flagship product of iGEN Biotech, is a genomic DNA extraction kit especially designed for low cell-count samples such as amniotic fluid and chorionic villa. The extraction system allows the derivation of high quality DNA from a small sample within a low assay time, delivering superior quality at a cost far lower than that of leading brands.

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Abstract

DNA Extraction from low-cellularity samples is a difficult task due to poor yields and long processing times, particularly when the downstream use is sensitive such as human DNA sequencing. A few examples of such types of low-cellularity samples are those used for prenatal diagnosis (i.e., amniotic fluid, chorionic villi).

Standard procedures for low-cellularity samples begin with cell amplification through a time-consuming *in vitro* culture necessary to raise a sufficient sample size to make subsequent analysis either by cytogenetics or molecular technologies, feasible. Thus, diagnosis using standard procedures may take several days, or even weeks. In high-risk pregnancies, such as in cases where there is risk of toxoplasmosis, waiting a few weeks may mean life and death.

Furthermore, cutting out *in vitro* culture time from the process translates into reduced man-hours and less likelihood of human error. It also means that automated systems are not overworked, and require shorter operating hours, and longer life cycles.

Most importantly, the quality and quantity of the yield generated by **iGENatal™** kit is equivalent to those generated by expensive automated systems.

About **iGENatal™**

IGEN's **iGENatal™** kit has been specifically designed for low cellularity samples and provides a rapid and easy extraction process that consists of 5 phases and requires the use of six different solutions. These phases are: lysis, isolation, precipitation, washing, drying and resuspension. The longest phase is lysis (30 – 90 minutes, depending on sample type), for a total assay duration of approximately 3 hours.

iGENatal™ kit is based on an organic-phase extraction (phenol/chloroform), with an optimized procedure due to cleaning and differentiating between phases and providing great efficiency to the phase extraction.

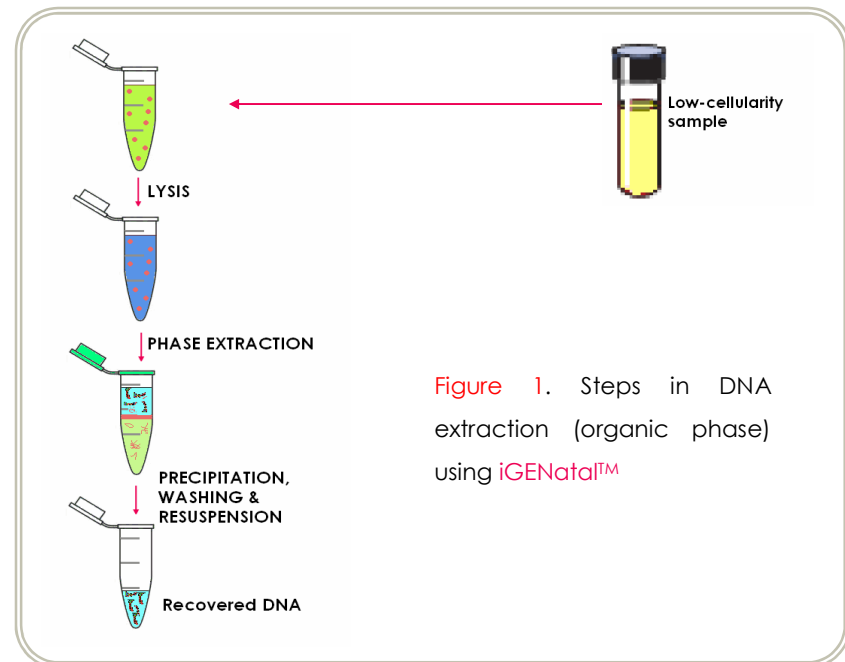


Figure 1. Steps in DNA extraction (organic phase) using **iGENatal™**

High yield from low cellularity

Current genomic extraction kits usually focus their action on whole blood, plasma, serum, mammalian or plant tissue, but there is no commercial kit targeting low cellularity samples. The iGENatal™ Kit can extract sufficient DNA for downstream sequencing use from as few as 50,000 cells, a yield only obtainable from expensive automatic systems.

Thus, yields of recovered DNA do not depend on the amount of samples available. From minimal starting amounts (2 ml of amniotic fluid or 2-3 mg of chorionic villi) high performance is guaranteed (approximately 100 µg/ml of DNA from amniotic fluid and 700-800 µg/ml of DNA from chorionic villi).

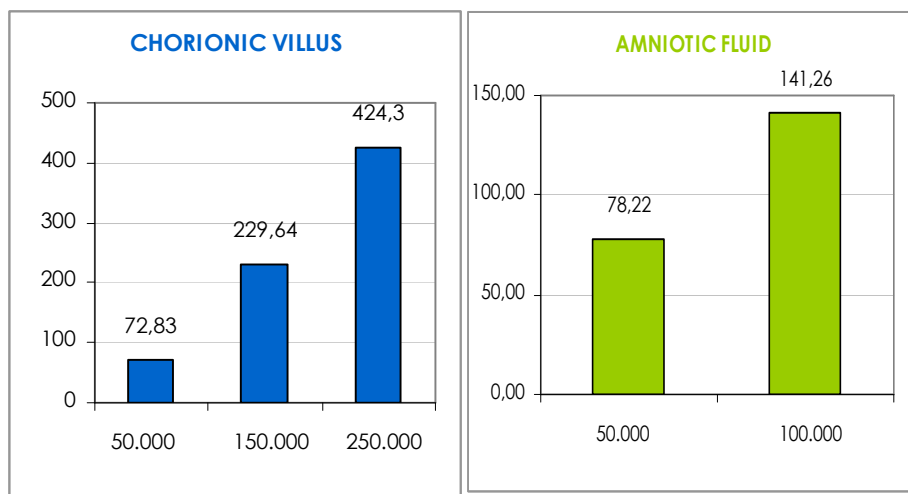


Figure 2. iGENatal™ Kit yield. Obtained DNA concentrations (ng/µL) from different cell concentrations from A) Chorionic Villi; B) Amniotic Fluid.

High quality and purity of obtained DNA

The optimization of the organic-phase separation procedure unique to the iGENatal™ kit provides DNA that is practically free of impurities. The most critical feature of this kit is that the recovered DNA is RNA- & peptide- free. The obtained DNA retains its high quality, unlike other kits in the market, such as silica columns which usually yield DNA that is contaminated with traces of nucleotides. Furthermore, this kit minimizes protein and other macro-molecules contamination. Absorbance ratios, A_{260}/A_{280} and A_{260}/A_{230} , are between 1.7 – 1.9 and over 2 respectively. A_{260}/A_{280} ratios between 1.7 – 1.9, which are obtainable using the iGENatal™ kit, implies low RNA and peptide contamination.

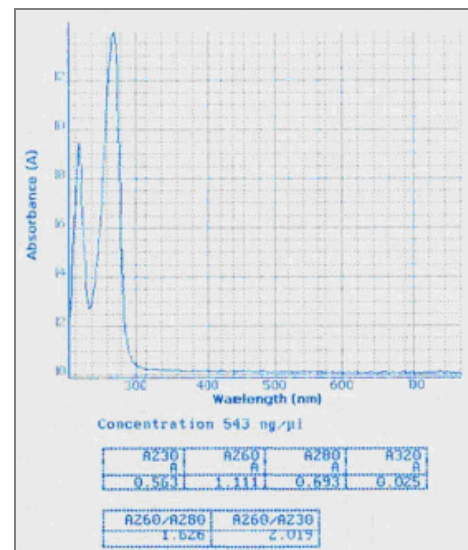


Figure 3. Ratios A_{260}/A_{280} y A_{260}/A_{230} . Absorbance spectrum of obtained genomic DNA from 2 mg of chorionic villi by iGENatal™ Kit.

Sequencing-grade DNA

Molecular biology is at constant evolution and requires better methods to perform at lower concentrations without losing quality, effectiveness and yield volume. iGENatal™ is perfectly adapted to the latest DNA analysis technologies. DNA obtained with this method can be analyzed by microarrays, q-PCR, a-CGH, X-MAP and all types of massive sequencing technologies due to very low DNA fragmentation.

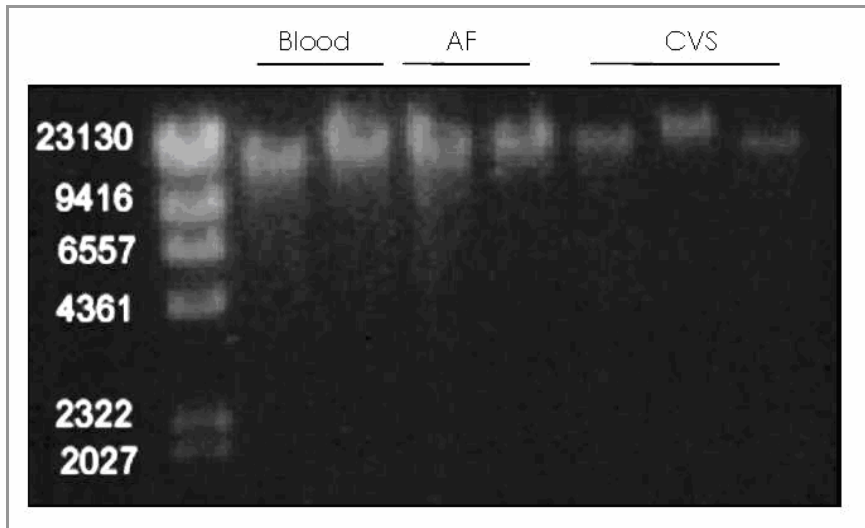


Figure 4. Agarose electrophoresis (0,8%) of genomic DNAs obtained from prenatal samples (amniotic fluid, AF; chorionic villi, CVS) using iGENatal™ compared with DNA from Blood using standard procedures.

Extraction-time optimization

Although the iGENatal™ Kit targets low-cellularity samples, it does not imply that it requires increased process time. The approximate duration for extraction is 90 minutes for amniotic fluid and 120 minutes for chorionic villi.

References

The kit it is being validated in several laboratories around Europe.

- ⇒ Wessex Regional Genetics Laboratory, Salisbury District Hospital. UK
- ⇒ Hospital San Joan de Deu. Barcelona, Spain
- ⇒ NE Thames Regional Genetics Service. Great Ormond Street Hospital NHS Trust. London, UK.
- ⇒ Quantitative Genomic Medicine Laboratories, SL. Barcelona, Spain.