QuickExtract™ DNA Extraction Solution and QuickExtract™ FFPE DNA Extraction Kit

1. What is the QuickExtract DNA Extraction Solution, and how does it work?

It is an optimized blend of reagents designed to lyse cells and tissues, and extract genomic DNA for PCR applications, without further purification steps. The QuickExtract procedure involves two simple heating steps after adding the sample (Figure 1). These heating steps destroy other cellular components, such as RNA and many proteins, while leaving intact genomic DNA ready for PCR. Although the procedure does not inactivate all PCR inhibitors, the dilution of the sample minimizes their effect in most amplification procedures.

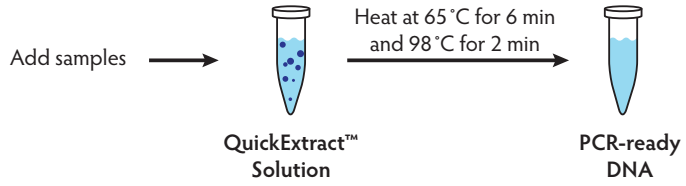

Figure 1. PCR-ready DNA in 8 minutes or less.

2. What is the QuickExtract FFPE DNA Extraction Kit, and how does it work?

This kit is similar to the QuickExtract DNA Extraction Solution, but the reagents have been optimized for the extraction of genomic DNA from formalin-fixed, paraffin-embedded tissue samples, without the need for toxic organic solvents such as xylene (Figure 2). The procedure requires only heat treatment to melt the paraffin, lyse the cells, decrease the formalin-induced cross-linking in the sample, and degrade compounds that may inhibit amplification.

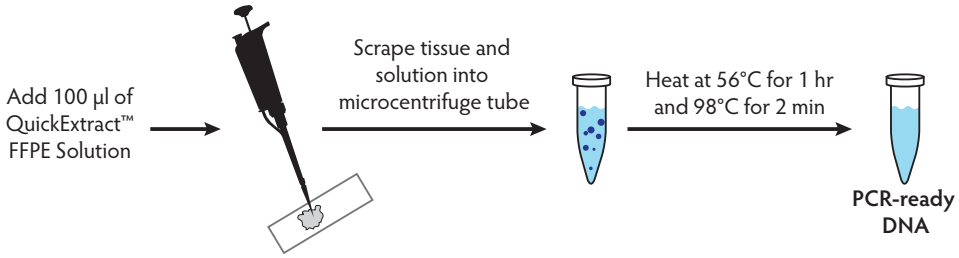

Figure 2. PCR-ready DNA from FFPE samples in an hour.

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3. Can I digest the extracted DNA with restriction enzymes or clone it?

No. The extracted DNA amplifies efficiently by PCR, but the extraction procedure does not remove proteins and other cellular debris; therefore, the DNA cannot be used for restriction digests or cloning. However, the resulting PCR product, amplified from a specific genomic DNA target, can be purified and restriction-digested or cloned.

4. What types of samples have been used successfully with QuickExtract products?

The QuickExtract DNA Extraction Solution has been used with a broad range of samples as referenced in hundreds of publications. Examples include bacteria, plant tissues, hair follicles, feathers (quill-end cells), tissue-culture cells, buccal cells, zebrafish (organs and scales), mouse tail snips, blood samples (liquid or Guthrie cards), and many more. The QuickExtract FFPE DNA Extraction Kit has been used with normal and tumor tissues from human and other mammalian samples.

5. How does the QuickExtract DNA Extraction Solution fit into a CRISPR-Cas9 gene editing workflow?

The speed and convenience of the QuickExtract procedure make it ideal for screening cells after transfection or transduction with vectors containing CRISPR-Cas9 sequences. Typically, genomic DNA is extracted from the cells using QuickExtract DNA Extraction Solution, and the success of the gene editing procedure is confirmed using PCR to detect the mutated sequences. The utility of the QuickExtract DNA Extraction Solution in these applications has been recognized by its incorporation into standard CRISPR-Cas9 gene editing protocols provided by Integrated DNA Technologies (www.idtdna.com/protocols; last accessed January 18, 2018).

6. Can I prepare a next-generation sequencing library from the extracted DNA?

Genomic DNA isolated with QuickExtract DNA Extraction Solution contains impurities that make it unsuitable for procedures other than PCR. Further cleanup (e.g., using Zymo DNA Clean and Concentrator™ Kits) is required before preparing sequencing libraries.

7. Can I use the extracted DNA in whole-genome amplification procedures, such as Phi-29 multiple-displacement amplification (MDA)?

Yes. Sorenson et al.[1] originally published a forensic application for QuickExtract-isolated DNA as a template for MDA, using the TempliPhi™ kit (GE Healthcare) and REPLI-g™ kit (Qiagen). For other representative examples, see Sills et al.[2] and Bushman et al.[3]

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8. What is the best way to quantify DNA isolated using the QuickExtract DNA Extraction Solution?

Because there is residual degraded RNA in the sample, using spectrophotometric methods (e.g., A_{260}) to quantify the DNA will give an artificially high estimate of the DNA concentration. The best method to quantify the DNA is by fluorimetry using a DNA-specific dye, such as Hoechst 332581 (bisbenzimide), or PicoGreen® dye (Thermo Fisher Scientific). These dyes bind specifically to double-stranded DNA and not to nucleotides, single-stranded DNA, or RNA.

References

1. Sorenson KJ et al. 2004. Whole-genome amplification of DNA from residual cells left by incidental contact. *Anal Biochem* **324**:312-314.
2. Sills ES et al. 2014. Determining parental origin of embryo aneuploidy: analysis of genetic error observed in 305 embryos derived from anonymous donor oocyte IVF cycles. *Mol Cytogenet* **7**:68.
3. Bushman DM et al. 2015. Genomic mosaicism with increased amyloid precursor protein (APP) gene copy number in single neurons from sporadic Alzheimer's disease brains. *eLife* **4**:e05116.

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