Manual

QuickExtract FFPE DNA Extraction Kit

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QuickExtract™ FFPE DNA Extraction Kit is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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Contents

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1. Introduction

The QuickExtract FFPE DNA Extraction Kit for formalin-fixed paraffin-embedded (FFPE) tissue provides a fast, simple and inexpensive method for preparing genomic DNA for PCR amplification from archival samples. QuickExtract DNA Extraction 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. Following heat treatment, the sample DNA is ready for PCR.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part number	Volume
QuickExtract FFPE DNA Extraction Kit	50 mL (500 reactions)	QEF81050	QuickExtract FFPE DNA Extraction Solution	SS000813-D1	50 mL

3. Product specifications

Storage: Store the QuickExtract FFPE DNA Extraction Solution at -20 °C in a freezer without a defrost cycle. Minimise the number of freeze/thaw cycles. Thawed extraction solution can be stored at 4 °C for 1 month or refrozen in small aliquots.

Quality control: The QuickExtract FFPE DNA Extraction Kit is function-tested by assaying for a PCR product from DNA extracted from a slide-mounted, FFPE tissue slice.

Contaminating activity assays: The QuickExtract FFPE DNA Extraction Solution is free of detectable RNase, exonuclease and endonuclease activities.

4. Notes on use of the QuickExtract FFPE DNA Extraction Kit

1. The yield of extracted DNA will vary by tissue type, size and preservation methods. Approximately 1-2 µg of DNA is obtained per square centimeter of tissue section.
2. Nucleic acids isolated from preserved, paraffin-embedded tissues are generally of poor quality. The degree of degradation of these samples limits analysis to mainly techniques involving amplification (see recommendations).
3. If extracted DNA will be used in Infinium HD FFPE genotyping and methylation arrays (Illumina, Inc.), the incubation step (56 °C) should proceed overnight.

5. Protocols

A. FFPE tissue slices from microscope slides

1. Add 100 µL of QuickExtract FFPE DNA Extraction Solution to the paraffin-embedded tissue section on the slide (0.8-1.0 cm^2 tissue section). Scrape with a sterile blade to remove the tissue section from the slide and carefully transfer the solution and tissue to a small microcentrifuge tube. Alternatively, the tissue section can be scraped and added to the solution in the tube, but prewetting the slide facilitates transfer of the tissue slice.

   Note: *If using a larger or smaller amount of tissue, adjust the reagent volume accordingly.*

2. Briefly centrifuge the tube to collect the solution and tissue at the bottom of the tube. If some tissue remains on the wall of the tube, begin heating the sample (Part A, Step 3) to melt the paraffin, then mix by vortexing and briefly centrifuge to collect the melted sample at the bottom of the tube.
3. Heat the tube in a thermocycler for 60 minutes at 56 °C, and then for 2 minutes at 98 °C.
   If desired, mix the sample by vortexing once during the incubation to aid in extraction, then briefly centrifuge the sample and continue the incubation.
4. Quantitate the DNA yield by fluorimetry to avoid an overestimation given by A_{260} readings.
5. Store the DNA at -20 °C, or at -70 °C for archival purposes.

B. Paraffin-embedded tissues

1. Remove a section of tissue using a clean microtome blade. Trim off any excess paraffin.
2. Place 10-50 mg of tissue or up to three 5-10 µm thick paraffin sections into a small microcentrifuge tube containing 100 µL of QuickExtract FFPE DNA Extraction Solution.
   Note: *The amount of extraction solution used can be adjusted to produce more concentrated extracted DNA. Thin slices are more important than the amount of tissue.*
3. Follow Part A, Steps 2 through 5 of the FFPE tissue slices from microscope slides protocol (above).

6. PCR amplification recommendations

1. 1-10 µL of extracted DNA can be used directly in standard and fast end-point PCR cycling profiles. Profiles should include 40 amplification cycles to ensure amplification.
2. Primers should be designed so that PCR amplicons will be less than 300 bases in length. The average size DNA that is extracted from FFPE tissues has been reported as 300- 400 bp.[1,2] Real-time PCR (qPCR) amplicons should be less than 200 bp in length. PCR assays should be well optimised for best results.
3. Extracted DNA has been used successfully in standard and fast end-point PCR, random amplification of polymorphic DNA (RAPD) PCR, mitochondrial PCR and qPCR. The resulting amplicons can be used for single-nucleotide polymorphism (SNP) detection or DNA sequencing.

7. References

1. Godfrey, T.I. *et al.*, (2000) *J. Mol. Diagn*. **2,** 84.
2. Lehmann, U. and Kreipe, H. (2001) *Methods* **25,** 409.

8. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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