truXTRAC® FFPE Total NA Auto 96 Kit
CONFIDENT AND CONSISTENT RESULTS
truXTRAC FFPE Total NA Auto 96 Kit

Robust, Reliable, High-quality, and High-throughput Extraction & Purification of Total Nucleic Acids from FFPE Tissue Samples

An ever-growing popularity of precision medicine initiatives renders data obtained from patient tissue samples as highly precious – from monitoring critical disease states to determining therapeutic regime.

Formalin Fixed Paraffin Embedded (FFPE) techniques have gained widespread popularity and are commonly used to preserve and archive solid tissues.

Critical Challenges

FFPE introduces several challenges to the researchers, such as, inefficient removal of paraffin, irreproducible and unreliable yield of analytes (DNA, RNA), lack of scalability, and many more.

- **Inefficient Deparaffinization**: Results in poor reproducibility and unreliable data
- **Working with Xylene**: Hazardous, requires a hood, and not easy to automate
- **Maximizing Yield of Analytes**: DNA and RNA at the same time, every time
- **Optimize Scalability**: Address a single or a thousand samples, with equal efficiency

Deparaffinization is a Critical Step

Passive deparaffinization methods often risk incomplete paraffin removal. When precious sample remains trapped in the wax, it is shielded from proteinase K (PK) digestion, resulting in inefficient extraction of analytes. Such inefficiencies can result in degraded, poor-quality nucleic acids and sub-optimal yields due to unwanted shearing and incomplete decrosslinking. Use of harsh organic solvents such as xylene and hexadecane, which are toxic, flammable, and require work to be completed in a vented hood challenges integrated workflows. Additionally, they require subsequent multiple time-consuming wash steps and complete drying. Integrating chemical deparaffinization into an automated workflow remains challenging and incomplete.
Adaptive Focused Acoustics® (AFA®) Technology and FFPE

Advances in clinical molecular pathology continue to grow fueling a stronger requirement for reliable FFPE extraction methods. Considering the value of the sample, differentially co-extracting high-yield and high-quality DNA and RNA provides a tremendous advantage. The truXTRAC FFPE kits are for efficient, hands-free paraffin removal, and high DV_{200} RNA scores. This offers an ideal solution to address challenges pertaining to low sample inputs. AFA enables the active removal of paraffin from FFPE tissue samples in an aqueous buffer, allowing simultaneous tissue rehydration.

AFA-enabled High-efficiency Extraction

The automated workflow with the truXTRAC FFPE Total NA Auto 96 Kit powered by AFA can increase DNA and RNA yields while minimizing sample degradation. Recovered high-quality nucleic acids are ideal for use in sensitive analytical methods such as next-generation sequencing or qPCR/RT-qPCR.

- User defined system setup maximizes laboratory efficiency
- Highly controlled, non-contact mechanical processing
- Eliminates need for columns and reduces wash steps
- Maintains sample integrity resulting in higher quality data

The Future of FFPE Tissue Analysis

Automate FFPE sample extraction workflows for higher throughput, quality, and consistency. Shown below is a proposed deck layout for Lynx (Dynamic Devices) Platform (Manual or Auto Transfer).
**The Complete High-Throughput Kit to Ease FFPE Nucleic Acid Extraction Workflows**

The truXTRAC FFPE Total NA Auto 96 Kit enables a comprehensive workflow comprising all reagents, consumables, and accessories necessary for the high-throughput extraction workflow. Along with time-saving convenience, users can be assured that all components work together for optimal performance, ensuring:

- High-throughput sample extraction and purification
- No harsh or toxic organic solvents
- Compatibility with FFPE scrolls and low mass inputs
- High-quality, high-yield nucleic acid recovery
- Processing up to 96 tubes per run

Only column A (front) with 8 truTUBEs is visual in these images.

1. Start with a FFPE scroll in the truTUBE arrayed in 96 Home and the 96 Shuttle
2. After addition of Rehydration Buffer (blue) and Deparaffinization Solution (colorless)
3. 96 Shuttle above 96 Home (rack) in transfer to 96 Heat Block
4. After incubation and centrifugation: tissue is in the blue phase, most wax in the clear phase
5. After aspiration and discard of upper layer (supernatant)
6. After addition of Tissue Lysis Buffer and AFA-enabled full deparaffinization, rehydration and homogenization of FFPE tissue

**Estimated Processing Time**

on the liquid handler is ~5 hours if RNA and DNA can be processed in parallel.
RNA Data

Yield and Purity

Yields of total RNA extracted from FFPE tissues were measured fluorescently (Qubit):

- Yields: truXTRAC FFPE total NA Auto 96 and parallel manual extraction (workflow/chemistry) are similar
- Yields: competitor kit lacks in two tissues (brain and breast tumor)

Purity was determined by measuring absorption at 230, 260, and 280 nm (Nanodrop):

- 260/280 & 260/230 nm ratios are similar between auto and manual truXTRAC extracted RNA Purity is satisfactory.
- RNA extracted with competitor kit lacks in purity as indicated by the low (< 0.8) 260/230 ratio of samples.
- Low 260/230 ratios in the lung tumor samples are explainable by the low yield (low concentration).

Amplifiability and Molecular Weight: Amplifiability of RNA (RT-qPCR) is an indication of integrity (de-crosslinking, average fragment length). truXTRAC auto and manual extracted RNA are both superior to the RNA extracted with the competitor kit. Quality is a better measure than quantity!

The $DV_{200}$ score is a direct measure of the molecular weight of RNA that is longer than 200 nucleotides. In most cases the $DV_{200}$ score can be manipulated by reducing the low MW RNA by tweaking the binding conditions during purification. $DV_{200}$ scores > 25 are generally acceptable for RNA-Seq.
DNA Data

Yield and Purity

Yields of DNA extracted from FFPE tissues were measured fluorescently (Qubit):

- Yields: truXTRAC FFPE total NA Auto 96 and a parallel manual extraction (workflow/chemistry) are similar
- Yields: competitor kit lacks significantly in two tissues (breast tumor and colon)

Purity was determined by measuring absorption at 230, 260, and 280 nm (Nanodrop):

- 260/280 nm and 260/230 nm ratios are similar between auto and manual truXTRAC extracted DNA as well as that extracted with the competitor kit.

Amplifiability and Molecular Weight: Amplifiability of DNA by the KAPA QC measures the ratio of amplicons of different length synthesized by PCR. The higher the score the better is the DNA quality (length, de-crosslinking etc.). KAPA QC values are similar across all experimental conditions.

Smear analysis of the extracted DNA (Fragment Analyzer) is an indication of the overall fragment length recovered. Note, that a high MW can also be an indication of lacking de-crosslinking. Lack of de-crosslinking can be easily detected when shearing the DNA with AFA energetics (examples on request).
**Impact of Suboptimal DNA Quality on DNA Shearing**

Note the lack of reproducibility in shearing of DNA extracted with the competitor kit. This could be due to incomplete de-crosslinking or impurities co-eluted with the DNA.

Fragment Analyzer electropherograms of DNA extracted from brain FFPE tissue before and after AFA-shearing (Covaris LE220-plus/ AFA-TUBE TPX).

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### Kit Components & Specifications

**truXTRAC FFPE Total NA Auto 96 Kit**

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<tr>
<th>Product Type</th>
<th>Product</th>
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<tr>
<td>Consumable</td>
<td>truTUBE™ Screw-Cap</td>
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<td>Consumable</td>
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### truXTRAC FFPE Total NA Auto 96 Accessory Kit

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**Sold Separately**

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<tr>
<td>Accessory</td>
<td>truTUBE Screw-Cap Prep Station</td>
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<tr>
<td>Reagent</td>
<td>Proteinase K Solution</td>
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</tr>
<tr>
<td>Reagent</td>
<td>Magnetic Bead Suspension</td>
<td>2.304 mL</td>
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### Necessary Equipment

The truXTRAC FFPE Total NA Auto 96 Kit was designed to fit your lab. Choose to automate every step, or only the steps that your lab is optimized for. For a fully-integrated workflow, you will need the following instruments and reagents:

- Covaris R or L Series Focused-Ultrasonicator
- Liquid Handler including reagent trays and pipette tips
- Automated Decapper for 96-Format SBS Racks
- Automation-compatible centrifuge for SBS Racks (e.g., Hettich....)
- 2 Liquid handler-integratable Dry Block Heaters (e.g., Q-instruments)
- Nuclease free water, ethanol and isopropanol

For more information on how to implement the truXTRAC FFPE Total NA Auto 96 Kit workflow into your laboratory, please contact our sales team or visit us at www.covaris.com/truxtrac-ffpe-na-auto-96-kits (Click link or scan QR code).