PrepFiler™ Forensic DNA Extraction Kit

Key Features

- Designed to improve the overall yield, concentration and purity of DNA isolated from both routine and challenging forensic samples.
- Provides high quality DNA, free from inhibitors of PCR, and suitable for downstream applications such as quantitative real-time PCR and PCR for short tandem repeat (STR) analysis.
- Specially developed magnetic particles and reagents optimize DNA binding and elution of DNA in a small volume.
- Offers streamlined protocols to process an extensive variety of forensic samples.
- Part of an integrated solution including novel reagents, consumables, and automated options to provide time-saving, cost-effective and reliable DNA purification.
- Improves downstream genotyping (STR analysis) success rate.

Introduction

The quantity and quality of genomic DNA extracted from a forensic sample can greatly impact the success of the sample analysis process and the overall quality of the final result. Forensic samples are among the most difficult specimens to process because they are often limited in quantity, may be environmentally exposed, and may require purification from difficult substrates containing PCR inhibitors. For laboratories looking to achieve better STR profile results, improve overall efficiency, and streamline the analysis process, maximizing the performance of the extraction method is one of the most effective ways to achieve these goals.

The PrepFiler™ Forensic DNA Extraction Kit is a breakthrough multi-component surface chemistry that achieves optimal DNA binding and highly efficient elution, enabling superior yield, inhibitor removal and automated capability for both routine and challenging forensic samples. Applied Biosystems has designed the PrepFiler™ kit specifically to improve the quantity and quality of DNA isolated from forensic samples, thereby increasing the potential to obtain probative information from downstream STR analysis.

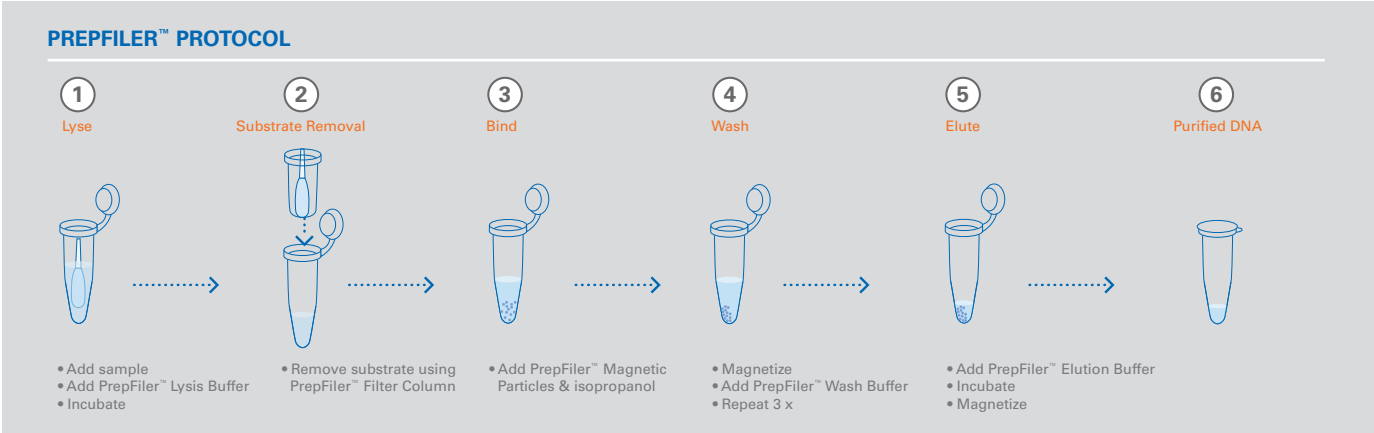

TABLE 1. PrepFiler™ kit workflow steps, kit components and novel features

Workflow Step	Kit Component	Novel Features
Lysis	PrepFiler™ Lysis Buffer	• Special formulation of detergents and chaotropic salts creates better lysis conditions for optimal liberation of DNA from the biological material. • Optimized protocols, with the option to use larger volumes of lysis solution, enable more efficient DNA extraction from a range of sample types and substrates, including highly absorbent material.
Substrate Removal	PrepFiler™ Filter Column (manual kit) PrepFiler™ Filter Plate (automated kit)	• Dedicated plastics designed to easily separate the substrate (swab, cloth, etc.) from the lysed sample. • Provided in single and 96-well formats.
DNA Binding	PrepFiler™ Magnetic Particles	• Polymer-embedded magnetic particles are much smaller in comparison to other commonly used magnetic extraction technologies, resulting in the following: - A larger surface area with higher DNA binding capacity - More easily distributed particles, which maximize the interface between the PrepFiler™ reagents and the magnetic particles, facilitating more effective DNA capture. • Uniquely designed reagent components, in combination with the composite structure of the magnetic particles, enable maximum DNA capture and stable DNA/particle complex formation.
Wash/Purify	PrepFiler™ Wash Buffer	• Specially formulated wash solution maximizes the removal of most PCR inhibitors while minimizing the loss of DNA during the wash step.
DNA Elution	PrepFiler™ Elution Buffer	• Combination of unique polymer-embedded magnetic particles and PrepFiler™ reagent components enable effective DNA release into the elution buffer. • Elution conditions have been optimized for maximum elution efficiency and recovery of purified DNA.

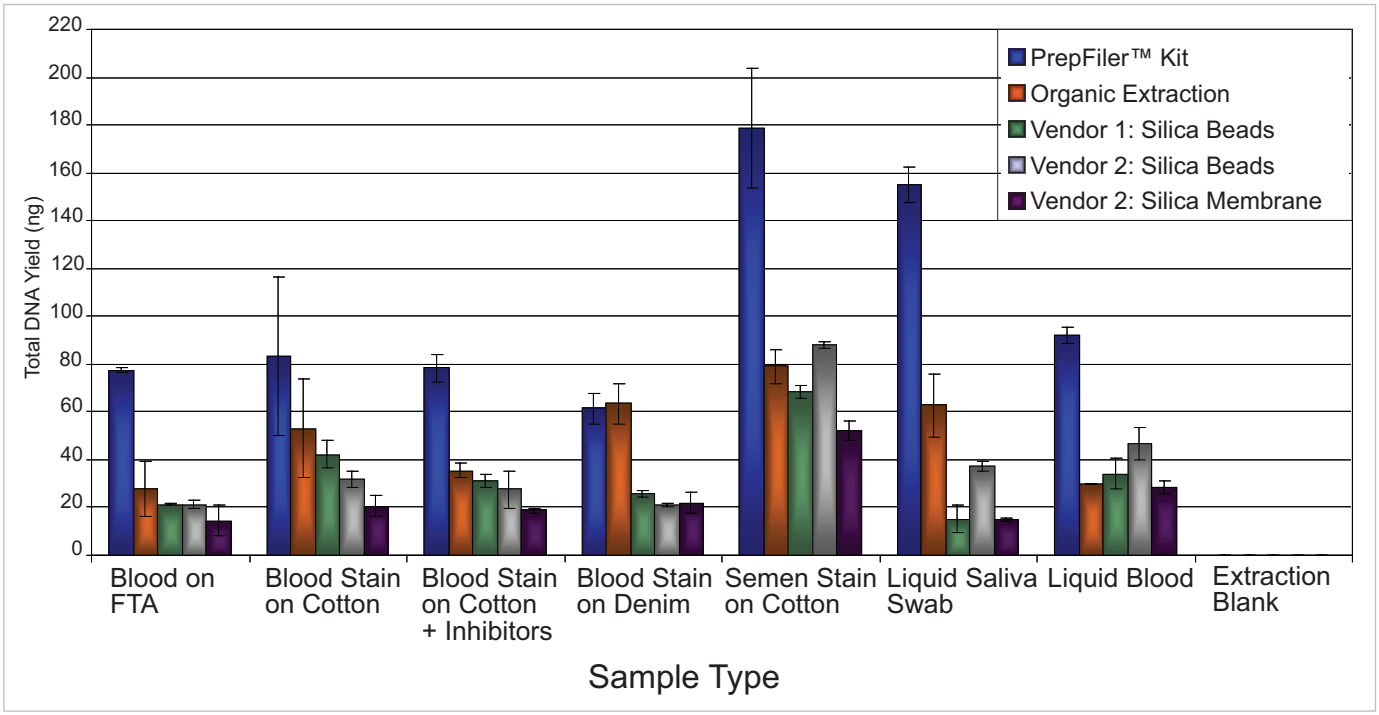

Figure 1. Quantifiler® Human kit results showing total yield of DNA isolated from simulated forensic casework samples (prepared from 2 µL blood, 50 µL saliva, and 1 µL semen) using the PrepFiler™ kit and 4 other methods commonly used in human identification laboratories. For all sample types investigated, the PrepFiler™ kit matched or exceeded the concentration and total yield of DNA produced by other methods. For certain sample types, the PrepFiler™ kit yielded greater than 100% more DNA than other methods tested.

Designed to Improve DNA Yield and Concentration

The PrepFiler™ kit uses a combination of uniquely structured magnetic particles and a multi-component surface chemistry optimized to maximize performance at each step in the extraction workflow (Table 1). This provides extremely efficient DNA binding capacity and high recovery of DNA concentrated in a small volume, making it suitable even for samples with minimal input.

As part of the validation process, Applied Biosystems tested the performance of the PrepFiler™ kit against a standard organic phenol/chloroform process and three other commercially available chemistries on a variety of forensic-type samples. The extracts were evaluated for DNA concentration, total quantity of DNA isolated and purity using the Quantifiler® Human DNA Quantification Kit. An example of the results is shown in Figure 1.

In all cases, the PrepFiler™ kit matched or exceeded the concentration and total yield of DNA produced by other methods, and, for certain sample types, the PrepFiler™ kit yielded greater than 100% more DNA than the other technologies tested. Increasing the yield of DNA isolated increases the likelihood of obtaining high quality DNA profiling results on the first attempt. This also provides the forensic scientist with more options in terms of the types and numbers of analyses that can be performed on each sample.

Effective Removal of PCR Inhibitors

The PrepFiler™ kit uses a specially formulated wash solution developed to maximize the removal of common PCR inhibitors found in forensic samples while minimizing any loss of DNA during the wash steps. To illustrate this, DNA was extracted from a number of samples containing inhibitors and then evaluated using the Quantifiler® Human DNA Quantification Kit. Subsets of the results are shown in Figure 2. All the extracts produced IPC C_T values comparable to the extraction blank and negative control samples, indicating that the inhibitors present in the original samples have been effectively removed by the PrepFiler™ kit during the extraction process. As a result, these samples produced clean, balanced DNA profiles which are easy to interpret.

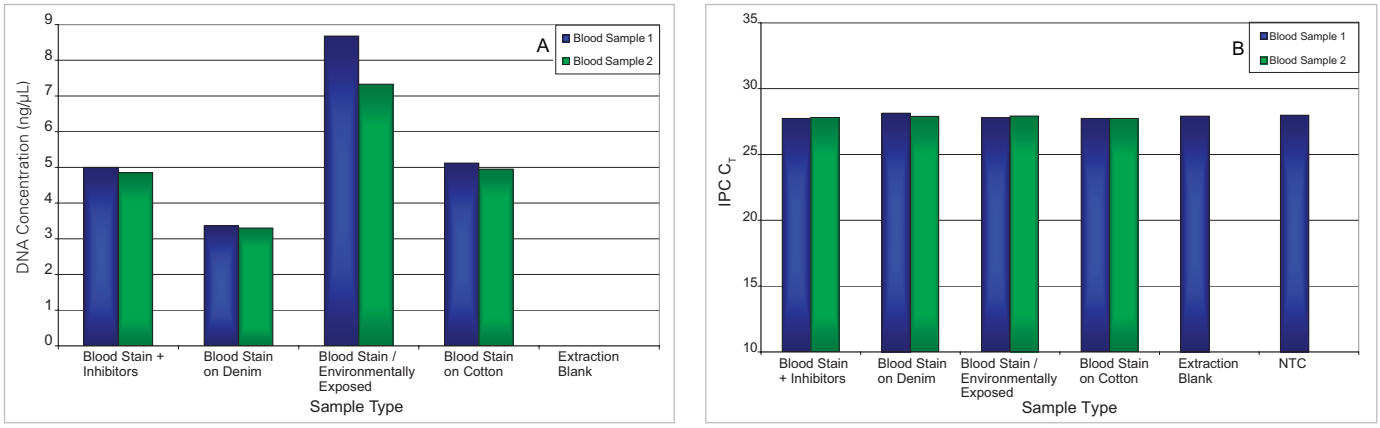

Figure 2. Quantifiler® Human kit results showing DNA Concentration (Panel A) and IPC C_T value (Panel B) results for two different blood samples (2 µL each) deposited on different substrates and exposed to different challenges (inhibitor mix includes hematin, humic acid, indigo and urban dust extract). All the extracts produced IPC C_T values comparable to the extraction blank and negative control samples, indicating that the inhibitors present in the original samples have been effectively removed by the PrepFiler™ kit during the extraction process.

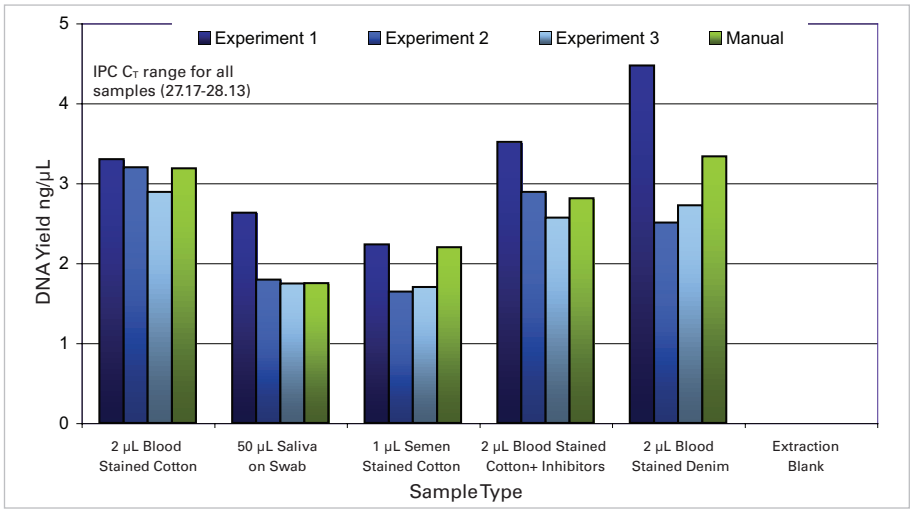

Figure 3. Quantifiler® Human kit data from 3 experiments where a selection of samples were extracted using the automated PrepFiler™ kit protocol (blue bars) and the manual PrepFiler™ kit protocol (green bars). Samples were processed in 8 and 3 replicates for automated and manual operations, respectively. For all samples extracted, the yield and performance of the automated protocol was consistent between experiments and was equivalent to or exceeded the performance of the manual protocol. The consistent results for the IPC C_T values (27.17-28.13) measured during the quantitation reaction indicate that the DNA was purified of any inhibitors present.

High Quality DNA from Multiple Sample Types

The PrepFiler™ Kit offers optimized protocols, with the option to use larger volumes of lysis solution, enabling more efficient DNA extraction from a wide range of sample types and substrates, including highly absorbent material. The PrepFiler™ kit is suitable for extraction of DNA from an extensive variety of forensic samples including liquid blood and saliva, buccal and surface swabs, body fluid stains, sexual assault samples, tissue samples (including formalin fixed specimens), hair, bones, teeth, fingernail scrapings, chewing gum, cigarette butts and samples on FTA paper. Most forensic sample types can be processed using a single, streamlined protocol. For certain types of challenging samples (such as teeth and bone samples), which require additional processing steps or procedural changes, suggested protocols are provided in the PrepFiler™ User Guide.

Automate Efficiently with Confidence

The PrepFiler™ kit's easy-to-use, magnetic particle technology is designed to adapt easily to automation on various liquid handling platforms. The kit is available in two package sizes, a smaller one for use with manual extraction protocols, and a larger one more suited to higher throughput, automated workflows.

To facilitate efficient automation of the extraction process, validated protocols, scripts and software for the PrepFiler™ kit on the Tecan Freedom EVO® 150 robotic platform have been developed for the HID EVOlution™- Extraction System. After lysis and substrate removal, the time required for processing on the HID EVOlution™- Extraction System is approximately 1.75 hours for 48 extractions, or 2.5 hours for 96 extractions. Relative performance of the automated and manual PrepFiler™ kits in terms of DNA concentration and removal of inhibitors is shown in Figure 3.

Improved Downstream Performance

Selecting an extraction method is one of the most important decisions a laboratory will make. The success of the extraction technique is pivotal in establishing a solid foundation for the rest of the workflow. The PrepFiler™ kit has been designed to satisfy the key requirements of a forensic DNA extraction method: flexibility of input sample type, delivery of high quantity and quality DNA, and suitability for manual and automated workflows. DNA extracted using the PrepFiler™ kit will increase data quality for a wide range of samples and facilitate improved downstream performance in the forensic sample analysis workflow.

PrepFiler™ Kit Formats and Components

The PrepFiler™ kit is available in manual and automated formats. Both formats are available with reagents only or with reagents plus the plastic components necessary for substrate removal, sample processing, and elution*.

The 100 preparation manual PrepFiler™ Forensic DNA Extraction Kit is available with PrepFiler™ Filter Columns for substrate removal and PrepFiler™ Spin Tubes for sample processing (Figure 4). A 6-tube Magnetic Stand is required for processing samples in the PrepFiler™ Spin Tubes or other microfuge tubes (Figure 5).

The 960 preparation PrepFiler™ Automated Forensic DNA Extraction Kit is available with PrepFiler™ Spin Plates and PrepFiler™ Filter Plates for sample lysis and substrate removal, and PrepFiler™ Processing Plates for sample processing (Figure 6). A 96-Well Magnetic Ring Stand is required for processing samples in a 96-well format (Figure 7).

*Elution tubes available only with the manual kit.

Figure 4. PrepFiler™ Spin Tubes and PrepFiler™ Filter Columns

Figure 5. 6-Tube Magnetic Stand

Figure 6. Assembled PrepFiler™ Spin and Filter Plate and PrepFiler™ Processing Plate

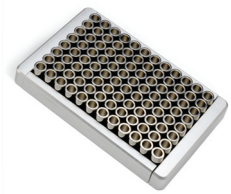

Figure 7. 96-Well Magnetic Ring Stand

PrepFiler™ Forensic DNA Extraction Kit
P/N 4392353

PrepFiler™ Forensic DNA Extraction Kit (with plastics)
P/N 4392852

PrepFiler™ Automated Forensic DNA Extraction Kit
P/N 4393135

PrepFiler™ Automated Forensic DNA Extraction Kit (with plastics)
P/N 4397977

MATERIALS PROVIDED WITH THE PREPFILER™ AUTOMATED FORENSIC DNA EXTRACTION KIT

Component	Description	Kit with Plastics P/N 4397977	Kit without Plastics P/N 4393135
PrepFiler™ Lysis Buffer	One bottle, 500 mL	Yes	Yes
PrepFiler™ Magnetic Particles	13 tubes, 1.5 mL	Yes	Yes
PrepFiler™ Wash Buffer Concentrate	One bottle, 500 mL	Yes	Yes
PrepFiler™ Elution Buffer	One bottle, 125 mL	Yes	Yes
PrepFiler™ 96-Well Spin Plate and Filter Plate	10 sets	Yes	No
PrepFiler™ 96-Well Processing Plates	10 plates	Yes	No

MATERIALS PROVIDED WITH THE PREPFILER™ FORENSIC DNA EXTRACTION KIT

Component	Description	Kit with Plastics P/N 4392852	Kit without Plastics P/N 4392353
PrepFiler™ Lysis Buffer	One bottle, 50 mL	Yes	Yes
PrepFiler™ Magnetic Particles	1 tube, 1.5 mL	Yes	Yes
PrepFiler™ Isopropanol	1 empty 60 mL bottle	Yes	Yes
PrepFiler™ Wash Buffer Concentrate	Two-100 mL bottles, each containing 26 mL of concentrate	Yes	Yes
PrepFiler™ Elution Buffer	One bottle, 12.5 mL	Yes	Yes
PrepFiler™ Filter Columns	100 filter columns	Yes	No
PrepFiler™ Spin Tubes	300 spin tubes	Yes	No

ORDERING INFORMATION

Description	P/N
PrepFiler™ Forensic DNA Extraction Kit (with plastics)	4392852
PrepFiler™ Forensic DNA Extraction Kit	4392353
PrepFiler™ Automated Forensic DNA Extraction Kit (with plastics)	4397977
PrepFiler™ Automated Forensic DNA Extraction Kit	4393135
6 Tube Magnetic Stand	AM10055
96-Well Magnetic Ring Stand	AM10050
PrepFiler™ Forensic DNA Extraction Kit User Guide	4390932
PrepFiler™ Automated DNA Extraction Kit Getting Started Guide	4393917
PrepFiler™ Forensic DNA Extraction Kit Quick Reference Card	4393918

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