One-Step Touch DNA Purification Kit

Touch DNA is defined as DNA collected from lost skin cells and other biological material left on touched surfaces by the papillary ridge patterns found on fingers, palms, toes, and soles. This evidence is extremely helpful in many criminal cases, from theft to sexual violence to murder. For example, in cases of murder or sexual assault, precious evidence could be gathered by studying the steering wheel of a vehicle used in thefts or weapons and clothing in cases of murder or sexual assault. Furthermore, collecting "Touch DNA" from a crime scene to identify a person of interest could be very valuable, especially in the absence of other types of biological evidence.

Typically, the DNA profiling process begins with the extraction of DNA from the substrate. DNA collection and recovery procedure remains the most critical in the DNA analysis process. Almost all experiments require an adequate amount of quantitative and qualitative DNA. As a result, the success of DNA typing is dependent on the availability of existing DNA templates. Better methods for recovering DNA are required, especially with touch samples containing a tiny amount of DNA. Various approaches have been utilized to increase evidential DNA's quantity and quality. However, depending on the extraction method and the quantification method's accuracy, the DNA extraction procedure can result in a loss of 20% to 90% of the initial template amount. The current purification technique utilized in forensic DNA cases is time-consuming and labor-intensive.

Furthermore, the column-based purification procedures result in DNA loss, preventing the successful typing of low copies or damaged samples. Direct PCR amplification has been proposed as one of the approaches for avoiding DNA loss from touch evidence samples. By skipping the extraction, quantification, and concentration steps, the majority of DNA can be retained. Laboratory employee error and DNA contamination from handling can be eliminated, and overall sample processing time and cost can be decreased. Although many DNA samples are extracted directly from the solid support, such as clothing, upholstery, paper, chewing gum, and cigarette butts, direct extraction can carry high concentrations of PCR inhibitors. Bioclone developed a revolutionary one-step DNA purification system based on magnetic beads to overcome these drawbacks.

BcMag™ One-Step Touch DNA Purification Kit uses novel Negative chromatography magnetic beads to quickly deliver higher quality and superior DNA yield from most trace touch samples. Those samples include body fluids, stains, swabs of body fluids, Strip removed cells, cigarette butts, Hair follicles, fingernail scrapings, epithelial cells, bite marks, semen, touch DNA samples, etc. The specially designed magnetic beads with our proprietary surface chemistry function simultaneously to lyse cells and capture the PCR inhibitors (Fig.1) once mixed with the sample. The magnetic beads-PCR inhibitor complex was then magnetically removed by a magnet while the pure DNA remaining in the solution was ready for downstream STR analysis. The purification kit provides a fast and simple method for DNA purification with only one tube, no liquid transfer, and no requirement for carrier RNA. It reduces the risk of DNA loss and carryover of extraction buffers from the traditional and tedious bind-wash-elute procedure. After preparing the lysates, it enables the processing of 96 samples in less than 15 minutes, with less than 1 minute of hands-on Time.

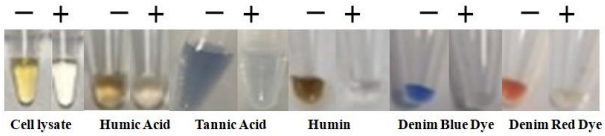

Fig.2 PCR inhibitor removal

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Principle and Workflow (Fig.2)

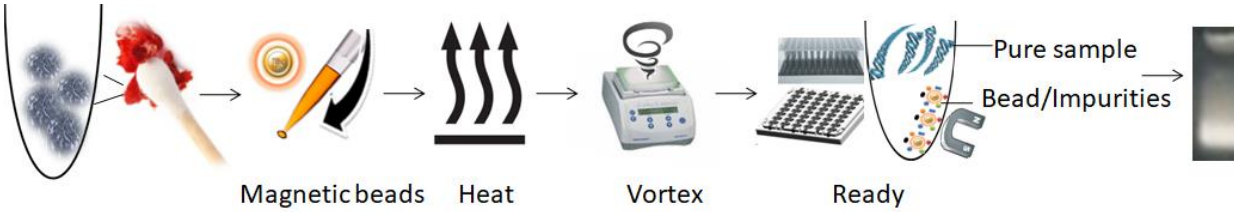

Fig.2 Principle and Workflow of touch DNA purification
1. Add functional magnetic beads to the sample.
2. Mix the samples with the magnetic beads and proteinase K to lyse the cells.
3. Mix by vertexing/pipetting for the beads to capture the PCR inhibitors.
4. Remove the beads with a magnet.
5. Aspirate the supernatant containing the pure ready-to-use DNA.

Performance

The purified DNA is suitable for use in sensitive downstream applications, such as PCR, qPCR, single-nucleotide polymorphism (SNP), short tandem repeat (STR) genotyping, genotyping, or next-generation sequencing (NGS).

Features and Advantages:

- Rapid and efficient purification protocol: without prior DNA isolation for subsequent use in direct workflows, No liquid transfer, and One-tube.
- Ultrafast: Process 96 samples in less than an hour.
- Highest nucleic acids recovery rates: Minimal loss of DNA during extraction
- Effectively removes inhibitors (Fig.2): polyphenolic compounds, humic/fulvic acids, acidic polysaccharides, tannins, melanin, heparin, detergents, denim dyes, divalent cations such as Ca^{2+}, Mg^{2+}, etc.
- Cost-effective: Eliminates columns, filters, laborious repeat pipetting, and organic reagents.
- High throughput: Compatible with many different automated liquid handling systems.

This product is intended for forensic, human identification, and paternity testing molecular biology applications. This product is not designed for disease diagnosis, prevention, or treatment.

Products

Components	Storage	50 preps, Cat # AS-101	100 preps, Cat # AS-102
BcMag™ U-DNA Beads	4°C	2.5 ml	5.0 ml
10x Lysis Buffer (100mM Tris-HCl, PH 9.0)	4°C	0.6 ml	1.2 ml
Proteinase K	-20°C	12.5 mg	25 mg
DTT(1M)	-20°C	15.4 mg	30.8 mg
Proteinase K Suspension Buffer	4°C	1.0 ml	2.0 ml

Shipping conditions: At ambient temperature

PROTOCOL

The following protocol is an example. The protocol can be scaled up or down as needed.

Notes

- DNA Yield: Varies (depends on sample size and type)
- DNA Size: Varies (depends on the quality of starting material
- Since there is no concentration step in the protocol, the concentration of the nucleic acid depends on the quality and quantity of the sample used.

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- Quantification of the nucleic acids: Use only fluorescence methods such as qPCR, Qubit, and Pico Green.
- OD260 methods such as Nanodrop and UV-spectrophotometry are not-suitable.
- For long-term storage, store the extracted nucleic acids at -20°C.

Materials Required by the User

Item	Source
Magnetic Rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag™ Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag™ Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag™ Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag™ Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag™ 96-well Plate Magnetic Rack.	• BcMa™ 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible Racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the Time and speed should be optimized, and the mixer should be: Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ** ***IMPORTANT!*** If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates has to be ≥2.5mm.	

Handling Samples (Table 1)

Follow these general guidelines when handling forensic samples:

- Cut the sample into small pieces where possible and appropriate to facilitate processing.
- Avoid overloading the sample tube to allow efficient mixing of Lysis Mix with the sample.
- When dealing with blood-stained items, ensure that the amount of blood processed is kept to a minimum (≤ 4 µl blood spot). Processing of larger, heavily blood-stained items may result in contamination of the purified DNA with heme.

Envelope flap	Up to 0.1×0.15-cm cutting
Chewing gum	Up to 5 mg (approximately 0.3×0.3×0.5-mm piece)
Cigarette butt, Lip Stickers	Swabbing for Cigarette butt, Lip stickers • If using a dry swab, use a sterile pipette to extract distilled water from the vial and apply 30µl to the side of the tip. Use no more than 30µl and do not immerse the swab in water. • Apply the fine tip to the cigarette filter paper area and rotate the swab with moderate pressure. Only rotate the specimen once to avoid compromising the sample by redepositing it. • Use a dry swab to collect the remainder of the specimen from the same spot. • Cut the swab tip with scissors and place it in a clean PCR tube.
Tape lifts	Up to 2.5 mm^2 cutting with saliva
surface swabs, saliva swabs	Up to 3 mg from the applied spot using scissors and forcipes.
Blood swabs	Up to 3 mg (equal to ≤ 4 µl blood spot) from the dried blood spot using scissors and forcipes.
Scurf (Dandruff, Furfur)	Up to 5mg
Hair follicles	Use 5 mg (3-5 hairs) of the root of the end of the human hair. Cut off the shaft 4 mm above the follicle.
Body fluids (saliva, semen, blood) on dye denim and other fabrics	Swabbing body fluids

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	• If using a dry swab, use a sterile pipette to extract distilled water from the vial and apply 30µl to the side of the tip. Use no more than 30µl and do not immerse the swab in water. • Apply the fine tip to the sample r area and rotate the swab with moderate pressure. Only rotate the specimen once to avoid compromising the sample by redepositing it. • Use a dry swab to collect the remainder of the specimen from the same spot. • Cut the swab tip with scissors and place it in a clean PCR tube.

B. Premix Beads solution Preparation

IMPORTANT!

1. Before pipetting, shake or Vortex the bottle to completely resuspend the Magnetic Beads.
2. Do not allow the magnetic beads to sit for more than 2 minutes before dispensing.
3. Proteinase K preparation: Provide protease K as lyophilized powder and dissolve at a 20 mg/ml concentration in Proteinase K Suspension Buffer. For example, 12.5 mg dissolved in 625 µl of Proteinase K Suspension Buffer. Divide the stock solution into small aliquots and store at -20°C. Each aliquot can be thawed and refrozen several times but should then be discarded.
4. DTT solution preparation: Provide DTT as powder and dissolve at a concentration of 1M in ultrapure water. For example, 15.4 mg dissolved in 100µl ultrapure water. It is stable for years at -20°C. Prepare in small aliquots, thaw it on ice, and use and discard. Store them in the dark (wrapped in aluminum foil) at -20°C. Do not autoclave DTT or solutions containing it. Avoid multiple freeze-thaw cycles.
5. Dilute DTT to a concentration of 10 mM from stock with ultrapure water and use it immediately. Discard unused *DTT* solution
6. Prepare a fresh Master Mix following Table 2 for the number of samples to be processed, plus 10% more (e.g., if you have 10 samples, prepare Master Mix for 11). Add the following components to the reservoir.

Table 2. Premix Beads solution

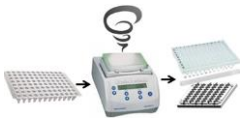
Component	One well (100 µL reaction volume)
BcMag™ U-DNA Beads	50 µL
10x Lysis Buffer	10 µL
Proteinase K (20mg/ml)	12.5 µL
DTT (10 mM)	3 µL
Sample	x
ULTRAPURE WATER	x
Total	100 µL

C. Isolation procedure

IMPORTANT!

- Pipet up and down premix beads solution in a reagent reservoir until the solution is homogeneous before dispensing.
- Do not allow the magnetic beads to sit for more than 5 minutes before dispensing.)

1. Transfer 100µl premix beads solution to the sample (except dye denim and other fabrics, whose purification goes to section D) to a new well of 96well PCR plate or 0.2ml PCR tube and add the sample.
2. Mix the sample well by Vortex or pipetting.
3. Place the PCR plate/tube into a thermocycler and incubate at:
   a. 65°C for 30 minutes
   b. 80°C for 10 minutes
4. Remove the PCR plate/tube from the thermocycler and then mix the sample with beads by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes (see picture).



5. Centrifuge at 3500 rpm for 5 minutes.

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6. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
7. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications. Using 1-5 ul in a 25µl RT-PCR or qPCR.

D. Isolation procedure for samples of dye denim and other fabrics

1. Transfer 100µl premix beads solution to the dye denim and other fabrics to a new well of 96well PCR plate or 0.2ml PCR tube and add the sample.
2. Mix the sample well by Vortex or pipetting.
3. Place the PCR plate/tube into a thermocycler and incubate at 60°C for 30 minutes.
4. Remove the PCR plate/tube from the thermocycler and place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
5. Rolling against the tube sides, press the sample against the side to squeeze as much of the liquid as possible and, simultaneously, leave beads as much as possible by forceps.
6. Remove the dye denim or fabrics and transfer supernatant with beads to a new PCR tube.
7. Place the PCR plate/tube into a thermocycler and incubate at 80°C for 10 minutes.
8. Remove the PCR plate/tube from the thermocycler, mix the sample with beads by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes (see picture).

9. Centrifuge at 3500 rpm for 5 minutes.
10. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
11. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications. Using 1-5 ul in a 25µl RT-PCR or qPCR.

E. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA/RNA Recovery	Poor starting sample material.	• Use better quality of the sample. • Add more samples
Ct value delays	Too many PCR inhibitors in the sample.	1. Add 25-50 µL BcMag™ U-DNA Beads to the extract solution and mix by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear. 2. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. Using 1-5 ul in a 25µl RT-PCR or qPCR. The sample is ready for downstream applications.
	Recovery DNA is so low.	• Use better quality of the sample. • Add more samples.

Related products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101

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One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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