One-Step Blood DNA Purification Kit

Isolation of DNA from blood and other body fluids in adequate quantities is integral to the clinic and forensic research and analysis. Large-scale clinical point-of-care tests require a quick and efficient DNA isolation technique for a trace amount of blood sample or other body fluids. However, current blood DNA purification protocols suffer drawbacks, including low yield, compromised quality, cost, tedious and time-consuming, toxic organic solvents, and many more. To overcome these drawbacks, we developed a novel revolutionary one-step DNA purification system based on magnetic beads and negative chromatography, which combines DNA extraction with removing all the PCR inhibitors from the samples without performing DNA isolation and purification steps.

BcMag™ One-Step Blood DNA Purification Kit allows rapid and efficient purification of genomic DNA from whole blood, serum, plasma, or other body fluids. The kit uses our unique proprietary magnetic beads to efficiently lyse cells and remove all impurities simultaneously in an aqueous buffer, leaving the DNA untouched. The procedure employs mild lysis conditions, avoiding harsh conditions such as alkaline lysis and toxic chemicals for lysing cells to maintain DNA integrity and the time-consuming cleanup of organic solvent from the sample. Furthermore, the magnetic beads eliminate PCR inhibitors (Fig.1) from samples in a single step without DNA extraction. It increases DNA integrity, boosts nucleic acid yields, and minimizes DNA loss caused by typical DNA purification techniques' time-consuming "bind-wash-elute" procedure. Following sample lysis, the straightforward one-step purification technique enables simultaneous processing of >96 samples and produces pure DNA in less than 30 minutes. Purified genomic DNA has the highest integrity and can be used in various downstream applications such as qPCR, STR, etc.

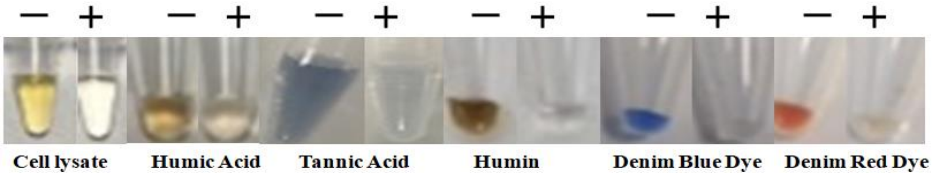

Fig.1

Workflow (Fig.2)

Fig.2 Workflow of one-step blood DNA Purification kit

The specially designed magnetic beads can quickly capture the impurity once mixed with the cell lysate. Then, the magnetic beads-impurity complex is magnetically removed by a magnet while the pure DNA remains in the solution.

1. Add magnetic beads to the sample.
2. Mix the samples with the magnetic beads and proteinase K and heat to lyse the cells.
3. Mix by vortexing/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 ready for downstream applications, such as PCR, qPCR, RT-PCR (reverse transcription-polymerase chain reaction), single-nucleotide polymorphism (SNP), short tandem repeat (STR) genotyping, genotyping, next-generation sequencing (NGS, veterinary genotyping, forensics, population studies, etc.

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.

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- Highest nucleic acids recovery rates: Minimal loss of DNA during extraction
- Effectively cell lysate cleanup and removes inhibitors: 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.

Handling and Storage: Store the kit components according to the table below on arrival.

Products

Components	Storage	50 preps, Cat # AF-101	100 preps, Cat # AF-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

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
- 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.**	

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A. Sample preparation

Handling Samples
Follow these general guidelines when handling forensic samples:

- When possible and appropriate, cut the sample into small pieces 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 to use a minimum sample (≤ 4 µl blood spot). Processing large, heavily blood-stained items may contaminate the purified DNA with heme.

Sample	Example sample input
whole blood	Up to 4µl
serum, plasma	5µl
other body fluids	20µl
Blood swabs	Up to 3 mg (equal to ≤ 4 µl blood spot) from the dried blood spot using scissors and forcipes.
Body fluids (saliva, semen, blood) on dye denim and other fabrics	Up to 3 mm^2 (cutting or punch) Blood sample, up to 3 mm^2 (cutting or punch, ≤ 4 µl blood spot) IMPORTANT: For the best results, punch in the center of the area where the sample was applied
storage cards	≤ 3m^2 (≤ 4 µl blood spot)

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 15 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.
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
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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