One-Step Mammalian Cell DNA Purification Kit

Large-scale purification of high-quality DNA from mammalian cells in sufficient quantities requires an efficient methodology for high sample volumes. Nevertheless, the protocols for most commercially available genomic DNA (gDNA) extraction kits suffer from a tedious binding-washing-elution step, poor integrity, DNA losses, and the use of toxic organic solvents. 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 Mammalian Cell DNA Purification Kit allows rapid and efficient purification of genomic DNA from cells. It uses novel negative selection chromatography magnetic beads to quickly capture impurities such as PCR inhibitors from cell lysate (See "PCR inhibitor removal"), leaving the DNA untouched. It reduces the risk of DNA loss and carryover of extraction buffers from the traditional and tedious bind-wash-elute procedure. The purification kit provides a fast and simple method for DNA extraction with only one tube, no liquid transfer, and no requirement for carrier RNA. 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.

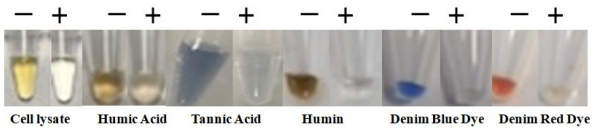

Fig.1 PCR inhibitor removal

Principle and Workflow (Fig.2)

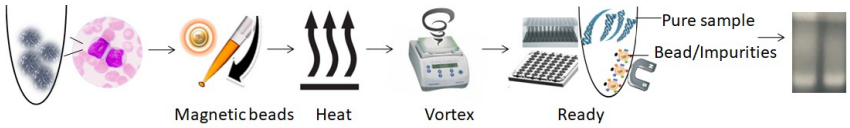

Fig.2 Principle and Workflow of mammalian cell DNA purification
1. Add functional magnetic beads to the sample.
2. Mix the samples with the magnetic beads and proteinase K to lyse the cells.
3. Mix by vertexing/pipetting for the beads to capture the PCR inhibitors.
4. Remove the beads with a magnet.
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), 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.
- Highest nucleic acids recovery rates: Minimal loss of DNA during extraction
- Effectively 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.

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Products
Handling and Storage: Please note that on arrival, the kit components should be stored according to the table below.

Shipping conditions: At ambient temperature

Components	Storage	50 preps, Cat # AA-101	100 preps, Cat # AA-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, make sure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates has to be ≥2.5mm.	

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.

Sample	Example sample input
Cells in Suspension	1. Centrifuge the suspension for 10 minutes at 800 × g 2. Remove all the liquid.
Adherent Cells	1. If the cells are in flasks, dislodge cells by the preferred method (Trypsin or cell scraper) and centrifuge suspension for 10 minutes at 800 x g. 2. Remove all the liquid.
Sample prep - Cell pellets	Cells can be collected directly into the extraction solution
Sample prep - FACS and LCM	Cells can be collected directly into the extraction solution
Sample prep - RNAlater™	1. Centrifuge suspension at 5000 x g for 5 minutes. 2. Remove all the liquid (a quick spin on the benchtop microcentrifuge can remove the last few drops). 3. wash with PBS buffer. 4. Centrifuge suspension at 5000 x g for 5 minutes 5. Remove all the liquid (a quick spin on the benchtop microcentrifuge can remove the last few drops). .

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

1. Resuspend the cell pellet (prepared from section A) with an appropriate amount of premix beads solution based on Table 3 in a new well of 96well PCR plate or 0.2ml PCR tube.

Table 3

Cell Numbers Pellet	Premix Beads solution (µl)
50,000 - 500,000	50-100
5,000 - 50,000	20-50
100 - 5,000	5-20
1 - 100	5

2. Mix the sample well by Vortex or pipetting.
3. Place the PCR plate/tube into a thermocycler and incubate at:

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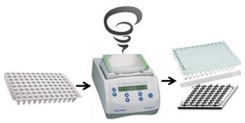
a. 65°C for 15 minutes
b. 80°C for 10 minutes

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 pictu



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 qPCR.

D. 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. 2.	1. Add 25-50 µL BcMag™ U-DNA Pure 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. The sample is ready for downstream applications. Using 1-5 ul in a 25µl RT-PCR or qPCR.
	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