Sexual Assault Casework DNA Purification Kit

Successful autosomal DNA analysis from sexual assault cases (also called Rape cases) requires a clean male DNA profile that the female DNA does not obscure. However, mixed DNA samples collected from female victims may contain only a trace level of sperm cells of the accused mixed with excessive female epithelial cells of victims. Hence, separating male DNA from female DNA is crucial in analyzing sexual assault cases. Differential lysis (also known as differential extraction) is the most popular approach for sperm DNA isolation in rape cases.

Differential lysis is based on the selective lysis and isolation of DNA from a mixture of sperm and epithelial cells. The epithelial cells in the mixture are lysed with lysis buffer without a dithiothreitol (DTT) reducing agent in the first step. The intact sperm cells remaining in the solution are collected by centrifugation. The sperm cells are lysed in the second step with the lysis buffer containing DTT. In the third step, the released DNA from the sperm cells is purified by phenol/chloroform extraction or silica membrane column. Although this method is very effective in separating these two cell fractions, the process is not only time-consuming and highly laborious but also causes a significant amount (60–90%) of male DNA loss at every step and results in the lack of reproducibility in sample preparation. BcMag™ sexual Assault Casework DNA Purification kit can overcome these technical hurdles.

BcMag™ Sexual Assault Casework DNA Purification Kit provides quick and efficient purification of male DNA from a trace amount of sexual assault samples. The kit uses novel negative selection chromatography magnetic beads to quickly capture impurities such as PCR inhibitors from cell lysate, leaving the male DNA untouched. It reduces the risk of DNA loss and buffers carryover from the time-consuming bind-wash-elute technique. The purification kit provides a quick and easy way to purify DNA. Quantitative PCR and STR analysis work well with pure DNA.

Features and Advantages

- Remove >95% epithelial cell DNA.
- High purity and recovery rate of sperm DNA from a variety of trace samples
- Rapid and efficient purification protocol: One tube, without prior sperm DNA isolation for subsequent use in direct workflows, and no liquid transfer.
- Effectively removes inhibitors (see picture “PCR inhibitor removal (Fig.1): 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

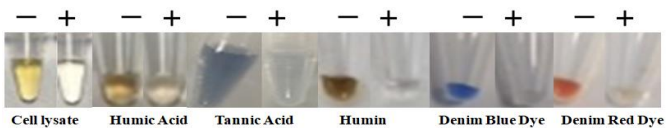

Fig.1 Magnetic PCR inhibitor removal

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Fig.2 Workflow of sexual Assault Casework DNA Purification

1. Add Lysis Buffer to break the epithelial cells and release their DNA.
2. Centrifuge to remove all epithelial cell DNA.
3. Add Lysis Buffer P to lyse the sperm cells to release sperm DNA.
4. Mix the lysate with the magnetic beads to capture the PCR inhibitors.
5. Remove the beads with a magnet and aspirate the supernatant containing only the pure, ready-to-use sperm DNA.

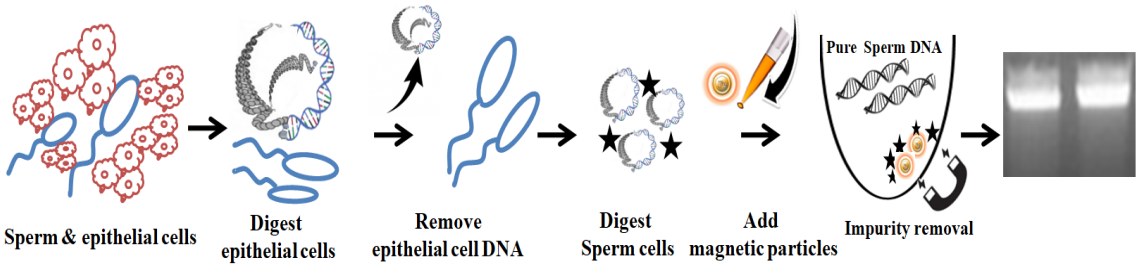

Fig.2

Handling and Storage: On arrival, the kit components should be stored according to the table below.

Components	Storage	50 preps, Cat # AT-101)	100 preps, Cat # AT-102
BcMag™ U-DNA Beads	4°C	1.5 ml	3.0 ml
5x Lysis Buffer E	4°C	4.0 ml	8.0 ml
10x Lysis Buffer P	4°C	250 µL	0.5 ml
Proteinase K (20mg/ml)	-20°C	30 mg	60 mg
DTT (1M)	-20°C	7.7 mg	15.4 mg
Proteinase K Suspension Buffer	4°C	1.5 ml	3.0 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, 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.

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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 (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
65°C Incubator chamber	
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 must be ≥2.5mm.	

A. Sample pretreatment to remove the epithelial DNA.

Sample Input

Sample	Example sample input
seminal /vaginal fluid mixture on fabric	25-mm^2 (cutting or punch)
Seminal/vaginal fluid mixture on the swab	Up to one swab

IMPORTANT!

1. 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.
2. 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.
3. Dilute DTT to a concentration of 10 mM from stock with ultrapure water and use it immediately. Discard unused DTT solution.

B. Procedure

1. Bring the thermal shaker temperature to 65°C.
2. Add the sample to a 1.5 ml centrifuge tube.
3. To the tube that contains the sample, add 400 µL lysis buffer E and 10 µl of Proteinase K.
4. **Note:** If multiple samples will be processed, scale up the volume of reagents used and prepare a master Lysis mix.

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5. Mix the sample by Vortex or invert the tube for 10-15 seconds.
6. Place the tube in a thermal shaker (or water bath), then incubate it at 65°C for 1 hour.
7. Use disposable tweezers to roll the sample against the tube sides, press the sample against the side to squeeze as much of the liquid as possible, and remove the sample.
8. Centrifuge at room temperature and 14,000 rpm for 5 minutes.
9. Remove the supernatant and save the sperm pellet.
10. **Repeat steps B3 to B8 twice to completely remove the epithelial DNA**.
11. Proceed to Purification of sperm DNA, next section.

C. Purification of sperm DNA.

Procedure

Premix Beads solution Preparation

IMPORTANT!

- Before pipetting, shake or Vortex the bottle to completely resuspend the Magnetic Beads.
- Do not allow the magnetic beads to sit for more than 2 minutes before dispensing.

1. 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	1 well /tube (50 µL reaction volume)
BcMag™ U-DNA Pure Beads	30µL
10x Lysis Buffer P	5µL
Proteinase K (20mg/ml)	6.25 µL
DTT (10 mM)	1.5 µL
ultrapure water	7.25 µL
Total	50 µL

2. To the PCR plate/tube that contains the sperm pellet, add 50 µL Premix Beads solution.
3. Mix the sample by Vortex or invert the tube for 10-15 seconds.
4. Place the PCR plate/tube into a thermocycler and incubate at:
    a. 65°C for 1 hour
    b. 80°C for 10 minutes
5. Remove the PCR plate/tube from the thermocycler and mix the sample with beads by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes.

6. Centrifuge at 3500 rpm for 5 minutes.
7. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
8. 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 for downstream applications.

D. Troubleshooting

Problem	Probable cause	Suggestion

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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™ 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. Using 1-5 ul in a 25µl STR 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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