MagListo™ Forensic Sample DNA Extraction Kit for *ExiPrep*™ 96 Lite

Ⅰ Before You Begin

1) Completely dissolve one vial of **Proteinase K** in **1,250 µl** of nuclease-free water. For short term storage, dissolved Proteinase K should be stored at **4°C**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20°C**.
2) Completely dissolve **Poly(A)** in **1,000 µl** of nuclease-free water. Divide the solution into aliquots of appropriate amount and store the aliquots at **–20°C**. Repeated freeze-thawing of the aliquots more than 3 times will result in the degradation of RNA.
3) Prepare 80% and 100% of additional ethanol (not provided).
4) **(Optional)** Prepare 1 M DTT (Dithiothreitol, not provided) for lysis of samples including hair, nail clipping or semen stains.

Ⅱ Sample Preparation

A. DNA Extraction from Buccal swab, hair, nail clipping or semen stain

1) Apply the forensic sample to a 1.5 ml tube.
2) Add **250 µl** of **TL Buffer** and **10 µl** of **Proteinase K** and mix thoroughly by vortexing.
3) **(Optional)** Add **20 µl** of **1 M DTT** and mix thoroughly by vortexing. **If the sample is hair, nail clipping or semen stains, this step is necessary to increase the yield.**
4) Incubate at **60°C** for **at least 1 hour**.
   (Note) Incubation time for complete lysis varies depending on the type of sample used and the age of starting materials. If the sample is nail clipping, bone or is considered old, extend incubation time up to overnight.
5) Add **250 µl** of **GB Buffer** and mix thoroughly by vortexing.
6) **(Optional)** Add **2 µl** of **dissolved Poly(A)** to improve extraction efficiency of DNA.
7) Incubate at **60°C** for **20 min**.
8) Centrifuge at 13,000 rpm for 1 min to obtain clear lysate.
9) Transfer **500 µl** of **lysate** to a new 96-well dome plate.

B. DNA Extraction from Small amount of Tissue

1) Disrupt (or homogenize) **the sample (~10 mg)** and place them in a 1.5 ml tube.
2) Add **90 µl** of **TL Buffer** and **10 µl** of **Proteinase K** and mix thoroughly by vortexing.
3) Incubate at **60°C until the sample is completely lysed.**
4) Add **100 µl** of **GB Buffer** and mix thoroughly by vortexing.
5) Transfer the lysate to a new 96-well dome plate.

C. DNA Extraction from Urine

1) Centrifuge the urine and discard the supernatant.
   a. **(~ 2 ml)** Centrifuge at 8,000 rpm (6,000 x *g*) for 2 min.
   b. **(~ 15 ml)** Centrifuge at 3,500 rpm (2,000 x *g*) for 10 min.

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2) Add **500 µl** of **1X PBS** and mix thoroughly by vortexing. Transfer the sample to a 1.5 ml or 2 ml tube.
3) Centrifuge at 8,000 rpm for 2 min and discard the supernatant.
4) Add **250 µl** of **TL Buffer** and **10 µl** of **Proteinase K** and mix thoroughly by vortexing.
5) **(Optional)** Add **10 µl** of **1 M DTT** and mix thoroughly by vortexing. **If the urine is expected to contain sperm cells, this step will improve yield.**
6) Incubate at **60°C** for **at least 1 hour**.
7) Add **250 µl** of **GB Buffer** and mix thoroughly by vortexing.
8) Incubate at **60°C** for **10 min**.
9) Transfer the lysate to a new 96-well dome plate.

D. DNA Extraction from Small Volumes of Blood and Saliva

1) Apply 1-100 µl of sample to a 1.5 ml tube.
2) Add **10 µl** of **Proteinase K**.
3) Add **100 µl** of **GB Buffer** and mix thoroughly by vortexing.
4) Incubate at **60°C** for **10 min**.
5) Transfer the lysate to a new 96-well dome plate.

III Loading the Kit to the Instrument

1) Add a **proper volume** of **100% Ethanol** to the 96-well dome plate containing lysate using multichannel pipette.

Lysate						100% Ethanol
Sample	TL Buffer	Proteiase K	GB Buffer	1 M DTT	Poly(A)	
Buccal swab/hair/nail clipping/ semen stain	250 µl	10 µl	250 µl	20 µl (optional)	2 µl (optional)	500 µl
Small amount of tissue	90 µl		100 µl	-	-	200 µl
Urine	250 µl		250 µl	10 µl (optional)	-	500 µl
Small volume of Blood and Saliva	-		100 µl	-	-	200 µl

2) Aliquot the solution from *MagListo*™ Forensic Sample DNA Extraction Kit to each of the new 96-well dome plate using multichannel pipette.

Cartridge No.	Solution	Volume
①	Lysate + Ethanol	Up to 1 ml
②	Magnetic NanoBeads solution	100 µl
③	WM1 Buffer	700 µl
④	W2 Buffer	700 µl
⑤	80% Ethanol	700 µl
⑥	80% Ethanol	700 µl
⑦	EA Buffer	100 µl

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3) Press the 'Plate' Button on the instrument.
4) Place the Magnetic Rod Cover to the Magnetic Rod.
5) Place the plate onto the proper position of the base plate.
6) Press the 'Standard Protocol' Button and select **'K-3603/K-3615 Genomic DNA (V1.0)'**.
7) Press the 'Run' Button to start the selected protocol.