MagListo™ 5M Forensic Sample DNA Extraction Kit (K-3615)

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**. Repeating of freeze-thawing of the aliquots more than 3 times will result in the degradation of RNA.

Ⅱ DNA Extraction from Forensic Sample

※ Handling samples

Dried body fluid spot or fingerprint (FTA card, papar, cloth, etc.): Punch out ~7 mm or cut out ~2 cm^2
Hair: Cut the hair 1 cm length from the hair root
Bone & teeth: Homogenize the bone or teeth ~100 mg
Chewing gum: Cut out ~30 mg
Cigarette butts: Cut out ~2 cm^2 from the end of the cigarette butt
Busccal swab: Cut the swab from its stick by hand or scissors

1) Apply the forensic sample to a 1.5 ml tube.
2) Add **300 µl** of **TL Buffer** and **10 µl** of **Proteinase K**, and mix thoroughly by vortexing.
(Optional) Add 20 µl of 1 M DTT and mix thoroughly by vortexing. If the sample is hair, nair clipping or semen stains, this step is necessary to increase yield.
3) Incubate at 60°C for at least 1 hour.
4) Add **300 µl** of **GB Buffer**, and mix thoroughly by vortexing and incubate at 60°C for 20 min.
(Optional) If the sample contains low number of cell (< $1x10^4$) or has a small amount of DNA (<100 ng), we recommend to add **2 µl** of dissolved **Poly(A)** solution to each sample after addition of GB Buffer.
5) Centrifuge at 13,000 rpm for 1 min and transfer the supernatant only into a new 1.5 ml tube.
6) Add **600 µl** of **absolute ethanol** to the tube containing the supernatant, and mix thoroughly by vortexing.
7) Add **100 µl** of **Magnetic NanoBead solution**, and mix thoroughly by vortexing.
(Note) Please vortex Magnetic NanoBead Solution well before use.
8) Place the tube in ***MagListo*™-2 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently 3~4 times.
9) Without removing the tube from *MagListo*™ rack, remove the supernatant.
10) Detach the magnet plate from *MagListo*™ stand. Add **700 µl** of **WM1 Buffer**. Mix thoroughly by vortexing.
11) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3~4 times until the beads bind tightly to the magnet.

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12) Without removing the tube from *MagListo*™ rack, remove the supernatant.
13) Detach the magnet plate from *MagListo*™ stand. Add **700 µl** of **W2 Buffer**. Mix thoroughly by vortexing, and repeat the above step 11~12.
14) Without removing the tube from *MagListo*™ rack, add **700 µl** of **WE Buffer** to "the opposite side of bead". Close the cap and gently invert the tube twice.
15) Discard the supernatant and completely remove the remaining supernatant by blotting.
16) Detach the magnet plate from *MagListo*™ stand. Add **30~80 µl** of **EA Buffer**. Mix thoroughly by vortexing and incubate at 60℃ for 1 min.
17) Vortex the tubes for 15 sec, then place them on the *MagListo*™ Magnetic Separation Rack. Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3~4 times until the beads bind tightly to the magnet.
18) Without removing the tube from *MagListo*™ rack, transfer the supernatant containing DNA carefully to a sterile microcentrifuge tube.

Ⅲ DNA Extraction from Small Amount of Tissue

1) Disrupt (or homogenize) **the sample (~10 mg)** then place them into a 1.5 ml tube.
2) Add **90 µl** of **TL Buffer** and **10 µl** of **Proteinase K solution**, mix thoroughly by vortexing and incubate at 60℃ until the sample is lysed.
(Optional) If RNA-free DNA is required, add up to 200 µg of RNase A (KB-0101, not provided) and incubate for 2 min at RT.
3) Add **100 µl** of **GB Buffer**, and mix thoroughly by vortexing.
4) Add **200 µl** of **absolute ethanol** and mix thoroughly by vortexing.
5) Go to **step 7** of "Ⅱ. DNA Extraction from Forensic Sample" in page 1 and continue extraction process.

Ⅳ DNA Extraction from Small Volumes of Blood and Saliva

1) Add **10 µl** of **Proteinase K solution** to a 1.5 ml tube.
2) Apply **1~100 µl** of **blood or saliva**.
(Note) If the sample volume is less than 100 µl, make the total volume 100 µl by adding 1X PBS (not provided).
3) Add **100 µl** of **GB Buffer**, mix thoroughly by vortexing and incubate at 60℃ for 10 min.
4) Add **200 µl** of **absolute ethanol** and mix thoroughly by vortexing.
5) Go to **step 7** of "Ⅱ. DNA Extraction from Forensic Sample" in page 1 and continue extraction process.

※ For more information, please visit www.bioneer.com and refer to the User's Guide of this kit.

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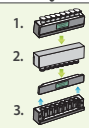
Before You Begin

1) Completely dissolve **Proteinase K** in **1,250 µl** of nuclease free water.
2) Completely dissolve **Poly(A)** in **1,000 µl** of nuclease free water.

Step	Image	Description
Lysis		Add Reagents according to sample type and resuspend the sample completely **1. Forensic samples** - FTA card, Paper, Cloth etc. ~2 cm^2, Hair 1 cm, Bone & Teeth ~100 mg, Chewing gum ~30 mg, Cigarette butts ~2 cm^2, Busccal swab - **Proteinase K 10 µl, TL Buffer 300 µl** **2. Small amount of tissue** - Sample ~10 mg - **Proteinase K 10 µl, TL Buffer 90 µl** **3. Small volumes of blood & saliva** - Sample 100 µl - **Proteinase K 10 µl, GB Buffer 100 µl**
		1. For forensic sample, 60°C for at least 1 hour **2. For tissue**, 60°C until the sample is completely lysed **3. For blood & saliva**, 60°C for 10 min
		**1. For forensic sample**, add **GB Buffer 300 µl**, mix and 60°C for 20 min **2. For tissue**, add **GB Buffer 100 µl** and mix
Precipitation		**1. For forensic sample**, centrifuge at 13,000 rpm for 1 min and transfer the supernatant into a new tube. Add **absolute ethanol 600 µl** and mix **2. For tissue**, add **absolute ethanol 200 µl** and mix **3. For blood & saliva**, add **absolute ethanol 200 µl** and mix
Binding		Add **Magnetic NanoBead 100 µl** and mix until the beads are fully resuspended
		Follow these 3 Magnet Attach/Detach Step 1. Attach magnet 2. Discard the supernatant 3. Detach magnet
1st Wash		Add **WM1 Buffer 700 µl** and mix until the beads are fully resuspended
		Repeat the above magnet attach/detach step (step **1, 2 and 3**)
2nd Wash		Add **W2 Buffer 700 µl** and mix until the beads are fully resuspended
		Repeat the above magnet attach step (step **1 and 2**)
3rd Wash		Add **WE Buffer 700 µl** to "the opposite side of the bead pellet" Close the cap and gently invert the rack twice
		Repeat the above magnet detach step (step **2 and 3**)
Elution		Add **EA Buffer 30~80 µl** and mix
		60°C Heating block for 1 min