MagListo™ cfDNA Extraction Kit (K-3619)

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) CL Buffer, CB buffer and CW1 buffer contain chaotropic salt. You should take the appropriate laboratory safety precautions and wear gloves when handling.
3) Prepare isopropanol.

Ⅱ Pretreatment of Samples

a. Plasma from whole blood
   1) Centrifuge the blood samples at 2,000 x *g* for 10 min.
   2) Transfer the supernatant to a new 50 ml tube.
   3) Repeat the above step 1.

b. Serum from whole blood
   1) Allow the blood samples to clot for 30 min ~ 1 hour at room temperature before centrifugation.
   2) Centrifuge the samples at 2,000 x *g* for 10 min.
   3) Transfer the supernatant to a new 50 ml tube.

c. Urine and Saliva
   1) Centrifuge the urine samples at 3,000 x *g* for 30 min.
   2) Transfer the supernatant to a 50 ml tube.

Ⅲ cfDNA Extraction from Plasma/Serum/Urine/Saliva

1) Transfer the collected samples **(~5 ml)** to a 50 ml tube.
2) Add **40 µl** of **Proteinase K** solution per 1 ml of sample and mix well.
3) Add **same sample volume** of **CL Buffer** to the collected sample tube.
4) Incubate at 60°C for 10 min.
5) Add **375 µl** of **isopropanol** per 1 ml of sample to the tube and mix well.
6) Add **200 µl** of **Magnetic NanoBeads solution** to the tube and mix thoroughly by vortex mixer until the beads are fully resuspended.
   (Note) Please vortex Magnetic NanoBeads solution well before use.
7) After incubation plate the tube for 5 min in the ice or 10 min at room temperature.
8) Place the tube in ***MagListo***™**-50 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently 3~4 times.
9) Without removing the tubes from rack, carefully pour the supernatant out and completely remove the remaining supernatant using paper towel by blotting.

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10) Detach the magnet plate from *MagListo*™ stand. Add **1 ml** of **CB Buffer** to the tube. Close the cap and mix by vortex mixer until the beads are fully resuspended.
11) Take the CB buffer mixed with magnetic nano beads and transfer into the new 1.5 ml or 2 ml tube.
12) Place the tube in ***MagListo*™-2 Magnetic Separation Rack** with the magnet plate attached and invert the rack 3~4 times gently until the beads bind tightly to magnet.
13) Without removing the tubes from rack, discard supernatant carefully and completely remove the remaining supernatant onto a paper towel by blotting down.
14) Detach the magnet plate from *MagListo*™ stand. Add **1 ml** of **CW1 Buffer**, close the cap and mix thoroughly by vortexing. Then repeat the above step 12-13.
15) Detach the magnet plate from *MagListo*™ stand. Add **1 ml** of **TA Buffer**, close the cap and mix thoroughly by vortexing. Then repeat the above step 12-13.
16) Detach the magnet plate from *MagListo*™ stand. Add **30 µl (1 ml) / 50 µl (3 ml) / 70 µl (5 ml)** of **EA Buffer** to the tube and resuspend by pipetting.
17) Incubate the tubes at 60℃ for 1 min and vortex the tubes for 15 sec.
18) Centrifuge at 1,000 rpm for 1 min.
19) Carefully transfer the supernatant containing cfDNA to a sterile tube.

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

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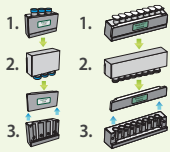
Before You Begin

1) Completely dissolve one vial of **Proteinase K** in **1,250 µl** of nuclease free water.
2) Prepare Isopropanol.

Step	Image	Description
Lysis		Add Reagents according to sample type **1. Serum, Saliva, Plasma, Urine** - Collected sample ~**5 ml** - **Proteinase K 40 µl** per 1 ml of sample - **CL Buffer** with **equal volume** to sample
		Incubate at 60°C for 10 min
Precipitation		Add **Isopropanol 375 µl** per 1 ml of sample and mix
Binding		Add **Magnetic NanoBead 200 µl** and mix until the beads are fully resuspended Incubate at RT for 10 min or on ice for 5 min
		Follow these 3 steps 1. Attach magnet 2. Discard the supernatant 3. Detach magnet
1st Wash		Add **CB Buffer 1 ml** and mix until the beads are fully resuspended
Transfer		Transfer into new 1.5 ml or 2 ml tube
		Repeat the above magnet attach/detach step (Step **1,2 and 3**)
2nd Wash		Add **CW1 Buffer 1 ml** and mix until the beads are fully resuspended
		Repeat the above magnet attach/detach step (Step **1,2 and 3**)
3rd Wash		Add **TA Buffer 1 ml** and mix until the beads are fully resuspended
		Repeat the above magnet attach/detach step (Step **1,2 and 3**)
Elution		Add **EA Buffer 30 µl (1 ml) / 50 µl (3 ml) / 70 µl (5 ml)** and mix