MagListo™ 5M Viral DNA/RNA Extraction Kit (K-3624G)

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 can 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) Dissolve **Poly(A)** with **500 µl** of **ER Buffer**. Gently mix with vortex mixer. Mix dissolved Poly(A) solution into **VB Buffer**. Shake it thoroughly.
3) Add correct amount of **absolute ethanol (not provided)** to **VWM1 Buffer** and **RWB2 Buffer,** respectively (see bottle label).
4) Pre-heat ER Buffer at 60°C before use.

II Viral DNA/RNA Extraction

1) Add **10 µl** of **Proteinase K** solution to a 1.5 ml or 2 ml tube.
2) Add **200 µl** of **Serum, Plasma** or **CSF** to the tube.
   (Note) Serum and Plasma can be used below **200 µl**.
3) Add **200 µl** of **VB Buffer** in the tube and mix by vortexing for 10 sec.
   To ensure efficient lysis, the sample should be mixed thoroughly with VB Buffer.
4) Incubate at **56-60°C** for **10 min**.
5) Add **400 µl** of **absolute ethanol (not provided)** and mix thoroughly by vortexing.
6) Add **200 µl** of **Magnetic NanoBeads Solution** to each tube and mix thoroughly by vortexing.
   (Note) Please vortex Magnetic NanoBeads solution well before use.
7) Place the tube in ***MagListo*™ -2 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently 3-4 times.
8) Without removing the tubes from rack, carefully discard the supernatant and completely remove the remaining supernatant using paper towel by blotting.
9) Detach the magnet plate from *MagListo*™ stand. Add **700 µl** of **VWM1 Buffer** to the tube. Close the cap and mix by vortexing or shaking until the beads are fully resuspended.
10) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3~4 times until the beads bind tightly to the magnet.
11) Without removing the tubes from *MagListo*™ rack, remove the supernatant.
12) Repeat the above step 9-11.
13) Detach the magnet plate from *MagListo*™ stand. Add **700 µl** of **RWB2 Buffer**. Mix thoroughly by vortexing, and repeat the above step 10-11.
14) Choose from two ways to remove residual ethanol.
    14-1. **(Washing Bead)** Without removing the tube from ***MagListo*™ rack with the magnet plate attached**, add **700 µl** of **WE Buffer** to "the opposite side of bead". Close the cap and gently invert the tube twice. Discard the supernatant and completely remove the remaining supernatant by blotting.
    (Note) Do not vortex or tap the mixture after adding WE Buffer, because vigorous mixing can reduce the yield of nucleic acid.

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14-2. **(Drying Bead)** Add **700 µl** of 80% **ethanol (not provided),** mix thoroughly by vortexing, and repeat the above step 10-11. Completely dry the beads with the tube open at 60℃ for at least 5 min. Remove the remaining supernatant by using pipette.

15) Detach the magnet plate from *MagListo*™ stand. Add **50-100 µl** of **ER Buffer**. Mix thoroughly by vortexing, and incubate the tube at 56-60℃ at least 1 min.

16) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

17) Without removing the tube from *MagListo*™ rack, transfer the supernatant containing nucleic acid carefully to a sterile microcentrifuge tube.

※ For more information, please visit www.bioneer.com and refer to the User 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) Dissolve **Poly(A)** with **500 µl** of **ER Buffer**. Gently mix with vortex mixer.
   Mix dissolved Poly(A) solution into **VB buffer** and shake it thoroughly.
3) Add correct amount of **absolute ethanol (not provided)** to **VWM1 Buffer** and **RWB2 Buffer,** respectively (see bottle label).
4) Pre-heat ER Buffer at 60°C before use.

Step	Image	Description
Lysis		Add **VB Buffer** to sample **(Sample type: Serum, Plasma, CSF)** - **Sample ~200 µl** - **Proteinase K 10 µl** - **VB Buffer 200 µl**
Incubation		60°C Heating block for 10 min
Precipitation		Add **absolute ethanol 400 µl** and mix
Binding		Add **Magnetic NanoBead 200 µl** and mix until the beads are fully resuspended
	1. 2. 3.	Follow these 3 steps Attach/Detach Step 1. Attach magnet 2. Discard the supernatant 3. Detach magnet
1st Wash		Add **VWM1 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 **VWM1 Buffer 700 µl** and mix until the beads are fully resuspended
		Repeat the above magnet attach/detach step (step **1, 2 and 3**)
3rd Wash		Add **RWB2 Buffer 700 µl** and mix until the beads are fully resuspended
		Repeat the above magnet attach step (step **1 and 2**)
4th Wash	(Washing Bead)	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**)
	(Drying Bead)	Add **80 % Ethanol 700 µl** and mix
		Repeat the above magnet attach/detach step (step **1, 2 and 3**) Dry the beads with the tube open at 60°C for at least 5 min
Elution		Add **ER Buffer 50-100 µl** and mix
		60°C Heating block at least 1 min