MagListo™ 5M Universal RNA Extraction Kit (K-3613)

Ⅰ Before You Begin

1) Add 10 µl β-mercaptoethanol (not provided) per 1 ml RD Buffer.
2) Prepare additional ethanol (80 % and 100 %) that is not included.

Ⅱ RNA Extraction from Cultured Cell

1) Centrifuge the cultured cells (10^4~10^6) for 5 min at 300 x *g*.
   Discard the supernatant carefully without disturbing the pellet.
2) Add **500 µl** of **RD Buffer** to the cell pellet and mix thoroughly by vortexing.
3) Add **300 µl** of **ethanol (96~100 %)** and mix immediately by using pipette.
4) Add **100 µl** of **Magnetic NanoBeads Solution** and mix thoroughly by vortexing.
   (Note) Please vortex Magnetic NanoBeads solution well before use.
5) Place the tube in ***MagListo*™ -2 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently 3~4 times.
6) Without removing the tubes from rack, carefully pour the supernatant out and completely remove the remaining supernatant using paper towel by blotting.
7) Detach the magnet plate from *MagListo*™ stand. Add **800 µl** of **RWM1 Buffer** to the tube. Close the cap and mix by vortexing or shaking until the beads are fully resuspended.
8) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3~4 times until the beads bind tightly to the magnet.
9) Without removing the tubes from *MagListo*™ rack, remove the supernatant.
10) Add **800 µl** of **RWA2 Buffer**, mix thoroughly by vortexing, and repeat the above step 8~9.
11) Choose from two ways to remove residual ethanol.
    11-1. **(Washing Bead)** Without removing the tube from *MagListo*™, 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.
    11-2. **(Drying Bead)** Add **800 µl** of **80 % ethanol**, mix thoroughly by vortexing, and repeat the above step 8~9. Completely dry the beads with the tube open at 60 ℃ for at least 5 min. Remove the remaining supernatant by using pipette.
12) Detach the magnet plate from *MagListo*™ stand. Add **50~100 µl** of **ER Buffer**.
    Mix thoroughly by vortexing, and incubate the tube at 60 ℃ for 1 min.
13) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3~4 times until the beads bind tightly to the magnet.
14) Without removing the tube from *MagListo*™ rack, transfer the supernatant containing RNA carefully to a sterile microcentrifuge tube.

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Ⅲ RNA Extraction from Plant Tissue

1) Add **500 µl** of **RD Buffer** to a maximum of 100 mg tissue powder and vortex vigorously.
2) Incubate at 60℃ for 1~3 min.
3) Centrifuge at full speed for 2 min.
4) Transfer the supernatant to a new microcentrifuge tube carefully without disturbing the pellet.
5) Add **300 µl** of **ethanol (96~100 %)** and mix immediately by using pipette.
6) Go to **step 4** of "RNA Extraction from Cultured Cell" in page 1 and continue the extraction process.

Ⅳ RNA Extraction from Animal Tissue

1) Homogenize the sample (20~30 mg) with a homogenizer, place them in a clean 1.5 ml tube, and add **500 µl** of **RD Buffer**.
2) Centrifuge the lysate at full speed for 3 min.
3) Transfer the supernatant, aqueous phase to a new microcentrifuge tube.
4) Add **300 µl** of **ethanol (96~100 %)** and mix immediately by using pipette.
5) Go to **step 4** of "RNA Extraction from Cultured Cell" in page 1 and continue the extraction process.

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

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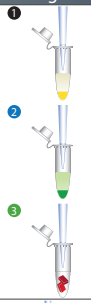
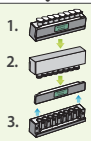
Before You Begin

1) Add 10 µl β-mercaptoethanol per 1 ml RD Buffer.
2) Prepare additional ethanol (80 % and 100 %) that is not included.

Step	Image	Description
Lysis		Add Reagents according to sample type **1. HeLa cell** - Cell pellet (~1x10^6) - **RD Buffer 500 µl** **2. Plant Tissue** - Tissue **~100 mg** - **RD Buffer 500 µl** - 60°C Heating block for 1~3 min **3. Animal Tissue** - Tissue **~20 mg** - **RD Buffer 500 µl**
Precipitation		Add **absolute ethanol 300 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 **RWM1 Buffer 800 µl** and mix until the beads are fully resuspended
		Repeat the above magnet attach/detach step (step **1, 2 and 3**)
2nd Wash		Add **RWA2 Buffer 800 µl** and mix until the beads are fully resuspended
		Repeat the above magnet attach step (step **1 and 2**)
3rd Wash	(Washing Bead) 	Add **WE Buffer 800 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 800 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 for 1 min