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

Ⅰ Before You Begin

1) Add 10 µl β-mercaptoethanol (not provided) per 1 ml of RD Buffer.
2) Prepare additional ethanol (80 % and 100 %) that is not included.
3) Add correct amount of **absolute ethanol (not provided)** to **RWMB1 Buffer** and **RWB2 Buffer,** respectively (see bottle label).

Ⅱ RNA Extraction from Cultured Cell

1) Centrifuge the cultured cells (10^4-10^6 cells) 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 **absolute ethanol (not provided)** 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 discard 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 **RWMB1 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 **RWB2 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 (not provided)**, 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) Grind ≤ 100 mg of plant sample in liquid nitrogen to a fine powder with a mortar and pestle and place them into an microcentrifuge tube.
2) Add 500 µl of RD Buffer to the sample and mix thorougly by vortexing.
3) Centrifuge at full speed for 2 min.
4) Incubate at 60℃ for 1-3 min.
5) Centrifuge at full speed for 2 min.
6) Transfer the supernatant to a new microcentrifuge tube carefully without disturbing the pellet.
7) Add **300 µl** of **absolute ethanol (not provided)** and mix immediately by using pipette.
8) 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 to a new microcentrifuge tube.
4) Add **300 µl** of **absolute ethanol (not provided)** 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 Guide of this kit.

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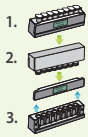
Before You Begin

1) Add 10 µl β-mercaptoethanol per 1 ml of RD Buffer.
2) Prepare additional ethanol (80 % and 100 %) that is not included.
3) Add correct amount of absolute ethanol (not provided) to RWMB1 Buffer and RWB2 Buffer, respectively (see bottle label).

Step	Image	Description
Lysis		Add Reagents according to sample type **1. HeLa cell** - Cell pellet (10^4-10^6 cells) - **RD Buffer 500 µl** **2. Plant Tissue** - Tissue **~100 mg** - **RD Buffer 500 µl** - 60℃ 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 **RWMB1 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 **RWB2 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 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 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℃ for at least 5 min
Elution		Add **ER Buffer 50-100 µl** and mix
		60℃ Heating block for 1 min