MagListo™ 5M Genomic DNA Extraction Kit (K-3603)

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 one vial of **RNase A** in **600 µl** of nuclease-free water. For short term storage, dissolved RNase A 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**.
3) If there is any precipitate in TL Buffer (Lysis), incubate on a heating block at 60°C.
4) Add correct amount of **absolute ethanol (not provided)** to **WM1 Buffer** (see bottle label).
5) Pre-heat EA Buffer at 60°C before use.

Ⅱ DNA Extraction from Whole Blood and Buffy Coat

1) Add **20 µl** of **Proteinase K** to 1.5 ml tube.
2) Apply **200 µl** of **whole blood** and **buffy coat** to the tube containing Proteinase K.
   (Note) If the sample volume is less than 200 µl, make the total volume 200 µl by adding 1X PBS (not provided).
3) Add **200 µl** of **GB Buffer** to the tube, mix thoroughly by vortexing, and incubate at 60°C for 10 min.
4) Add **400 µl** of **absolute ethanol (not provided)** and mix thoroughly by vortexing.
5) Add **100 µl** of **Magnetic NanoBead Solution** and mix thoroughly by vortexing.
6) Place the tube in ***MagListo*™-2 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently, 3-4 times.
7) Without removing the tube from rack, remove the supernatant.
8) Detach the magnet plate from *MagListo*™ stand. Add **500 µl** of **WM1 Buffer**. Mix thoroughly by vortexing.
9) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.
10) Without removing the tube from *MagListo*™ rack, remove the supernatant.
11) Repeat the above step 8-10.
12) Detach the magnet plate from *MagListo*™ stand. Add **700 µl** of **W2 Buffer**. Mix thoroughly by vortexing, and repeat the above step 9-10.
13) 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.
14) Discard the supernatant and completely remove the remaining supernatant by blotting.
15) Detach the magnet plate from *MagListo*™ stand. Add **100 µl** of **EA Buffer**. Mix thoroughly by vortexing, and incubate at 60°C for 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 DNA carefully to a sterile microcentrifuge tube.

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Ⅲ DNA Extraction from Cultured Cell

1) Centrifuge the cultured cells (~**1x10^6** cells) for 10 min at 300 x *g*.
2) Resuspend the pellet in **200 µl** of **1X PBS** and place them to 1.5 ml tube.
3) Add **20 µl** of **Proteinase K** to the tube.
4) Add **10 µl** of **RNase A**. Incubate for 2 min at RT.
5) Add **200 µl** of **GB Buffer**, mix thoroughly by vortexing, and incubate at 60℃ for 10 min.
6) Add **400 µl** of **absolute ethanol** (not provided) and mix thoroughly by vortexing.
7) Add **100 µl** of **Magnetic NanoBead Solution** and mix thoroughly by vortexing.
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.
12) Without removing the tube from *MagListo*™ rack, remove the supernatant.
13) Go to **step 12** of "Ⅱ. DNA Extraction from Whole Blood and Buffy Coat" in page 1 and continue the extraction process.

Ⅳ DNA Extraction from Animal Tissue

1) Disrupt (or homogenize) the sample (~**25 mg**) then place them into 1.5 ml tube.
2) Add **180 µl** of **TL Buffer** to the tube.
3) Add **20 µl** of **Proteinase K** and mix thoroughly by vortexing.
4) Add **10 µl** of **RNase A** and incubate for 2 min at RT.
5) Incubate at 60℃ until the tissue is completely lysed.
(Note) To increase the DNA purity, centrifuge the lysate at 13,000 rpm for 5 min and transfer the supernatant to 1.5 ml tube.
6) Add **200 µl** of **GB Buffer** and mix thoroughly by vortexing.
7) Add **400 µl** of **absolute ethanol (not provided)** and mix thoroughly by vortexing.
8) Add **100 µl** of **Magnetic NanoBead Solution** and mix thoroughly by vortexing.
9) Place the tube in ***MagListo*™-2 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently, 3-4 times.
10) Without removing the tube from rack, remove the supernatant.
11) Detach the magnet plate from *MagListo*™ stand. Add **700 µl** of **WM1 Buffer**. Mix thoroughly by vortexing.
12) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.
13) Without removing the tube from *MagListo*™ rack, remove the supernatant.
14) Go to **step 12** of "Ⅱ. DNA Extraction from Whole Blood and Buffy Coat" 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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Ⓥ DNA Extraction from Bacterial Cells (Gram-Negative Bacteria)

1) Harvest up to 1 x 10^9 bacterial cells by centrifugation at 6000 x *g* for 10 minutes to pellet cells. Discard the supernatant carefully with a pipette.
2) Resuspend the cell pellet in 180 µl of TL Buffer by vortexing or pipetting. Transfer the cell suspension into the clean tube.
3) Go to **step 3** of "IV. DNA Extraction from Animal Tissue" in page 2 and continue the extraction process.

Ⓥ DNA Extraction from Bacterial Cells (Gram-Positive Bacteria)

1) Harvest up to 1 x 10^9 bacterial cells by centrifugation at 6000 x *g* for 10 minutes to pellet cells. Discard the supernatant carefully with a pipette.
2) Resuspend the cell pellet in 180 µl of lysis buffer (not provided) by vortexing or pipetting. Transfer the cell suspension into clean tube.
   (Note) Lysis buffer for gram-positive bacteria can be prepared by using this formulation:
   20 mM Tris-HCl (pH 8.0), 2 mM sodium EDTA, and 1.2% Triton® X-100.
3) Add 20 µl of lysozyme (100 mg/ml, not provided) and mix well by vortexing.
4) If RNA-free genomic DNA is required, add up to 10 µl of RNase A to the sample and gently mix.
5) Incubate at 37°C for 30 minutes.
6) Add 20 µl of Proteinase K and mix well by vortexing.
7) Add 200 µl of GB Buffer and mix well by vortexing.
8) Incubate at 60°C for 30 minutes or until bacterial cells are completely lysed.
9) Go to **step 7** of "IV. DNA Extraction from Animal Tissue" in page 2 and continue the extraction process.

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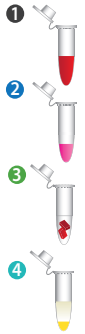
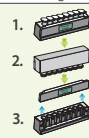
Step	Image	Description
Lysis		Add Reagents according to sample type **1. Whole blood and Buffy coat** - Sample **200 µl** - **Proteinase K 20 µl, GB Buffer 200 µl** **2. Cultured cell** - **1X PBS 200 µl** + Cell pellet (~**1x10^6**) - **Proteinase K 20 µl, RNase A 10 µl, GB Buffer 200 µl** **3. Animal tissue** - Disrupt sample ~**25 mg** - **Proteinase K 20 µl, TL Buffer 180 µl, RNase A 10 µl** **4. Bacterial cells (Gram-Negative, Positive Bacteria)** - ~1x10^9 cells - Gram-Negative: **TL Buffer 180 µl, Proteinase K 20 µl, RNase A 10 µl** - Gram-Positive: **Lysis Buffer 180 µl, Lysozyme 20 µl, RNase A 10 µl, Proteinase K 20 µl, GB Buffer 200 µl**
Incubation		60°C Heating block for 10 min (**For Animal tissue and Bacterial cells, incubate at** , 60°C Heating block until it is completely lysed)
Precipitation		Add **absolute ethanol 400 µl** and mix (**For Animal tissue and Gram-Negative Bacteria, add GB Buffer 200 µl and absolute ethanol 400 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 (**For Whole blood**, 1st washing with **WM1 Buffer 500 µl twice**)
		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 attaching 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 detaching step (step **2 and 3**)
Elution		Add **EA Buffer 100 µl** and mix
		60°C Heating block for 1 min