MagListo™ Genomic DNA Extraction Kit for *ExiPrep*™96 Lite

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 **RNase A** in **600 µl** of nuclease-free water. For short term storage, dissolved RNase A should be stored at **4°C**.

Ⅱ Sample Preparation

A. DNA Extraction from Cultured Cells

1) Harvest cultured cells (10^4-10^6 cells) by centrifugation at 300 x *g* for 5 min to pellet cells. Discard the supernatant carefully without disturbing a cell pellet.
2) Completely resuspend the cell pellet in **200 µl** of **1x PBS buffer**.
3) Add **20 µl** of **Proteinase K** to the sample.
4) Add **10 µl** of **RNase A** to the sample, mix thoroughly, and incubate for 2 min at room temperature.
5) Add **200 µl** of **GB Buffer** to the sample and mix immediately by vortexing.
6) Incubate at 60°C for 10 min.
7) Transfer the lysate to a new 96-well dome plate.

B. DNA Extraction from Whole Blood and Buffy Coat

1) Apply 200 µl of whole blood or buffy coat sample to a clean 1.5 ml tube.
2) Add **20 µl** of **Proteinase K** to the sample.
3) Add **200 µl** of **GB Buffer** to the sample and mix immediately by vortexing.
4) Incubate at 60°C for 10 min.
5) Transfer the lysate to a new 96-well dome plate.

C. DNA Extraction from Animal Tissue

1) Homogenize 25 – 50 mg of fresh or thawed animal tissue sample with a homogenizer and place them into a clean 1.5 ml tube.
2) Add **200 µl** of **TL Buffer** and **20 µl** of **Proteinase K** and **RNase A** to the sample and mix thoroughly by vortexing.
3) Incubate at 60°C until the sample has been completely lysis (1 hour).
4) Add **200 µl** of **GB Buffer** to the lysate and mix immediately by vortexing.
5) Transfer the lysate to a new 96-well dome plate.

D. DNA Extraction from Bacterial Cells (Gram-Negative Bacteria)

1) Harvest up to $1x10^9$ bacterial cells by centrifugation at 8,000 rpm for 5 min to pellet cells. Discard the supernatant carefully with a pipette.
2) Completely resuspend the cell pellet in **180 µl** of **TL Buffer** by vortexing or pipetting.
3) Add **20 µl** of **Proteinase K** and **RNase A** and mix thoroughly by vortexing.

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4) Incubate at 60℃ for 1 hour or until bacterial cells are completely lysed.
5) Add **200 µl** of **GB Buffer** to the sample and mix immediately by vortexing.
6) Transfer the lysate to a new 96-well dome plate.

E. DNA Extraction from Bacterial Cells (Gram-Positive Bacteria)

1) Harvest up to 1x10^9 bacterial cells by centrifugation at 8,000 rpm for 5 min to pellet cells. Discard the supernatant carefully with a pipette.
2) Completely resuspend the cell pellet in **180 µl** of **Lysis Buffer** (for Gram-Positive bacteria, not provided) by vortexing or pipetting.
(Note) Lysis Buffer for Gram-Positive bacteria: 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1.2% Triton® X-100
3) Add **20 µl** of **lysozyme** (**100 mg/ml**, not provided) and **10 µl** of **RNase A** and mix thoroughly by vortexing.
4) Incubate at 37℃ for 30 min.
5) Add **20 µl** of **Proteinase K** and mix immediately by vortexing.
6) Add **200 µl** of **GB Buffer** and mix immediately by vortexing.
7) Incubate at 60℃ for 30 min or until bacterial cells are completely lysed.
8) Transfer the lysate to a new 96-well dome plate.

Ⅲ Loading the Kit to the Instrument

1) Add **400 µl** of **100 % Ethanol** to the 96-well dome plate containing lysate using multichannel pipettes.
2) Aliquot the solution from *MagListo*™ Genomic DNA Extraction Kit to each of the new 96-well dome plate using multichannel pipette.

Cartridge No.	Solution	Volume
①	Lysate + Ethanol	Up to 830 µl
②	Magnetic Nano Bead solution	100 µl
③	WM1 Buffer	700 µl
④	WM1 Buffer	700 µl
⑤	W2 Buffer	700 µl
⑥	80 % Ethanol	700 µl
⑦	EA Buffer	100 µl

3) Press the 'Plate' Button on the instrument.
4) Place the Magnetic Rod Cover to the Magnetic Rod.
5) Place the plate onto the proper position of the base plate.
6) Press the 'Standard Protocol' Button and select **'K-3603/K-3615 Genomic DNA (V1.0)'**.
7) Press the 'Run' Button to start the selected protocol.