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One page protocol

AccuPrep® Genomic DNA Extraction Kit (K-3032)

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℃**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20℃**.
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℃**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20℃**.
3) Add correct amount of **absolute ethanol** to **WA1 Buffer.**

Ⅱ DNA Extraction from Whole Blood and Buffy Coat

1) Add **20 µl** of **Proteinase K** to a 1.5 ml or 2 ml tube.
2) Apply **200 µl** of **whole blood** or **buffy coat** to the tube containing Proteinase K.
3) Add **200 µl** of **GB Buffer** to the sample and mix immediately by vortex mixer.
4) Incubate at **60℃** for **10 min.**
5) Add **400 µl** of **absolute ethanol** and mix well by pippetting.
6) Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a collection tube) without wetting the rim.
7) Close the tube and centrifuge at **8,000 rpm** for **1 min.**
8) Discard the solution from the collection tube and reuse the collection tube.
9) Add **500 µl** of **WA1 Buffer** without wetting the rim, close the tube, and centrifuge at **8,000 rpm** for **1 min.**
10) Discard the solution from the collection tube and reuse the collection tube.
11) Add **500 µl** of **W2 Buffer** without wetting the rim, close the tube, and centrifuge at **8,000 rpm** for **1 min.**
12) Discard the solution from the collection tube and reuse the collection tube.
13) Centrifuge once more at **13,000 rpm** for **1 min** to completely remove ethanol, and check that there is no droplet clinging to the bottom of Binding column tube.
14) Transfer the Binding column tube to a new 1.5 ml tube for elution, add **50~200 µl** of **EA Buffer** onto Binding column tube, and wait for at least 1 min at RT (15~25℃).
15) Centrifuge at **8,000 rpm** for **1 min** to elute.

Ⅲ DNA 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) Resuspend the pellet in **200 µl** of **1X PBS.**
3) Add **20 µl** of **Proteinase K.**
4) Add **10 µl** of **RNase A**, mix thoroughly and incubate the tubes for 2 min

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at room temperature.

5) Go to **step 3** of **"DNA Extraction from Whole Blood and Buffy Coat"** in page 1 and follow the instructions accordingly.

Ⅳ DNA Extraction from Animal Tissue

1) Homogenize the sample (25~50 mg) with a mortar and pestle, place them in a clean 1.5 ml tube, and add **200 µl** of **TL Buffer.**
2) Add **20 µl** of **Proteinase K** and **10 µl** of **RNase A.** Mix by vortexing.
3) Incubate at **60°C** for **1 hour**, or until the tissue is completely lysed.
4) Add **200 µl** of **GB Buffer**, and mix by vortexing.
5) Go to **step 5** of **"DNA Extraction from Whole Blood and Buffy Coat"** in page 1 and continue the extraction process.

Ⅴ DNA Extraction from Gram-Negative Bacteria

1) Collect the bacterial cells ~1 x 10^9 by centrifuging at **8,000 rpm** for **5 min.** Discard the supernatant (media) by using a pipette.
2) Add **180 µl** of **TL Buffer** to the collected cell pellet and completely resuspend by vortexing or pipetting. Transfer the cell suspension to 1.5 ml or 2 ml tube.
3) Add **20 µl** of **Proteinase K** and **10 µl** of **RNase A**. Mix by vortexing.
4) Incubate at **60°C** for **1 hour.**
5) Add **200 µl** of **GB Buffer**, and immediately mix by vortex mixer.
6) Go to **step 5** of **"DNA Extraction from Whole Blood and Buffy Coat"** in page 1 and continue the extraction process.

Ⅵ DNA Extraction from Gram-Positive Bacteria

1) Collect the bacterial cells ~1x10^9 by centrifuging at **8,000 rpm** for **5 min.** Discard the supernatant (media) by using a pipette.
2) Add **180 µl** of **Lysis Buffer** for Gram-Positive bacteria (not provided) to the collected cell pellet and completely resuspend by vortexing or pipetting. Transfer the cell suspension to 1.5 ml or 2 ml tube.
(Note) Lysis Buffer for Gram-Positive bacteria : 20 mM Tris-HCl (pH8.0), 2 mM EDTA and 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 the tubes at **37°C** for **30 min.**
5) Add **20 µl** of **Proteinase K.**
6) Add **200 µl** of **GB Buffer** and mix thoroughly by vortexing.
7) Incubate the tubes at **60°C** for **30 min** or until bacterial cells are completely lysed.
8) Go to **step 5** of **"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's Guide of this kit.