PrimePrep™ Genomic DNA Extraction Kit (from Tissue)

Introduction

PrimePrep™ Genomic DNA Extraction Kit (from Tissue) is desinged for isolating genomic DNA from mammalian cells, tissues and bacteria cells (Gram$^-$).
This kit process does not require mechanical homogenization, so total hands on work time is only less 30 minutes.

Storage Conditions and Product Stability

All solutions should be tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.
The Proteinase K solution (20 mg/ml) should be stored at - 20 ℃.

Kit Components

Reagents / Cat.No.	K-3000 (50 prep.)	K-3001 (200 prep.)
Spin column	50 ea	50 ea x 4
Collection tube	100 ea	100 ea x 4
Buffer TL	20 ml	20 ml x 4
Buffer GB	12 ml	12 ml x 4
Buffer GW1	20 ml	20 ml x 4
Buffer GW2	10 ml	10 ml x 4
Buffer GE	10 ml	10 ml x 4
Proteinase K Sol. (20 mg/ml)	1.2 ml	1.2 ml x 4

Before you begin

1. Add 15 ml ethanol to Buffer GW1 before use.
2. Add 40 ml ethanol to Buffer GW2 before use.
3. Check Buffer TL, GB and GW1 before use for salt precipitation.

Note: Redissolve any precipitation by warming to 50 ℃.
Do not shake Buffer TL vigorously.

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Experimental Protocol

1. for Tissue Cut up to 20 mg of tissue and transfer into a microcentrifuge tube (not provided) and add 200 µl of Buffer TL.
   If used tissue has a higher number of cell (e.g. spleen or liver), reduce the starting material to 10 mg.
   for Gram⁻ Bacterial Cell Harvest the overnight cultured cell 100 ~ 200 µl by centrifuge at 13,000 rpm for 30 sec. and discard the supernatant.
   Add 200 µl of Buffer TL and resuspend the cell pellet.
2. Add 20 µl of Proteinase K solution, mix by vortexing, and incubate at 56℃ until the tissue is completely lysed.
   Vortex accasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform. Lysis time varies depending on the type of tissue processed.
   Overnight lysis dose not influence the preparation.
3. Spin down the tube briefly to remove any drops from inside of lid.
4. Optional RNase A treatment If RNA-free genomic DNA is required, add the 20 µl of RNase A Solution (10 mg/ml).
5. Add 200 µl of Buffer GB to the sample. Mix well by pulse-vortexing for 15 sec.
6. Incubation at 56℃ for 10 minutes.
7. Add of 200 µl absolute ethanol and mix well by pulse-vortexing for 15 sec.
   After this step, briefly spin down to get the drops clinging under the lid.
8. Carefully transfer the lysate into the upper reservoir of the spin column (fit in a 2 ml tube) without wetting the rim.
9. Centrifuge at 10.000 rpm for 1 min.
10. Transfer the spin column to a new 2 ml collection tube for filtration.
11. Add 500 µl of Buffer GW1 to the spin column, and centrifuge at 10,000 rpm for 1min.
12. After centrifugation, discard the flowthrough and transfer the spin column to a new 2 ml collection tube.
13. Add 500 µl of Buffer GW2 to the spin column, and centrifuge at 10,000 rpm for 1min.
14. After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.
15. Centrifuge once more at 12,000 rpm for 1~2 min to completely remove ethanol, and check that there is no droplet clinging to the bottom of collection tube.
    Residual GW2 in the spin column may cause problems in later application.
16. Transfer the spin column to a new 1.5 ml tube (not provided) for elution. Add 200 µl of Buffer GE onto spin column, and wait for at least 1 min at room temperature.
17. Elute DNA by centrifugation at 10,000 rpm for 1 min.

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