

IDPURE™ Spin Column Animal Tissue Genomic DNA Purification Kit

IDBB2614 100 Reactions

Kit Contents

Components	Size
ACL Solution	40 ml
PBS Solution	40 ml
AB Solution	40 ml
Proteinase K	40 mg
Wash Solution	24 ml
Elution Buffer	10 ml
Spin Column	100
2.0 ml Collection Tube	100

- ACL Solution may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37°C.
- Before use, add 2ml of sterilized water to the tube containing 40 mg of Proteinase K, keep at -20 °C for long term storage.
- Before use, add 96 ml of 100% ethanol to 24 ml Wash Solution. For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of Wash Solution = 4:1).
- Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 10% lower.

Storage: With the exception of the Proteinase K, the kit may be stored at room temperature. The proteinase K should be stored at 4°C for short term. The kit is stable for 12 months at room temperature. For longer storage, keep all contents cold.

Principle:

This kit is designed for fast isolation of genomic DNA from animal tissues. The kit contains a membrane embedded in spin column for binding up to 10 µg of genomic DNA. Nucleotides, proteins, salts, and other impurities do not bind to the Column. Purified genomic DNA can be applied in most molecular biology experiments including restriction digestion, Southern-blotting etc.

Applications:

Genomic DNA purification from different animal tissues.

Features:

- Preparation of high quality genomic DNA from variable sources.
- Rapid and economical.
- High yields
- No phenol / chloroform extraction , no ethanol precipitation

Procedure for Isolation of Genomic DNA from Variable Sources.

For Animal Tissue

- Cut up to 30 mg tissue and place in a 1.5 ml centrifuge tube.
- Add 300 µl of ACL Solution (Animal Cell Lysis Solution) to 1.5 ml centrifuge tubes and 20 µl Proteinase K.
- Incubate at 55 °C until the tissue is completely lysed (usually 1-3 hours). Occasionally vortex. Incubation in shaking water bath can reduce lysis time.
- Cool to room temperature. Vortex for 20 seconds and Centrifuge 12000 rpm for 5 minutes.
- Pipette 300 µl of supernatant into a spin column (if pellet not visible, repeat previous step) and add 300 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes.

6. Centrifuge 4000 rpm for 2 minutes and discard the flow-through.
7. Add 500 µl of Wash Solution, and spin at 8,000 rpm for 1 minute.
8. Repeat washing step 7.
9. Discard flow-through. Spin at 10,000 rpm for an additional minute to remove residual amount of Wash Solution.
10. Place the column into a clean 1.5 ml microfuge tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate the tube at 37 or 50 °C for 2 minutes. Incubation at 37 or 50 °C could increase recovery yield.
11. Spin at 10,000 rpm for 1 minute to elute DNA from the column.
12. Measure DNA quantity by UV absorption at A₂₆₀ (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel. Isolated genomic DNA should not contain RNA. Its length should be over 50 kb.

For Rodent Tail

1. Place numbered 1.5 ml centrifuge tubes in dry ice.
2. Cut 0.5 cm to 1 cm from end of tails and place in tubes.
3. Add 300 µl of ACL Solution to 1.5 ml centrifuge tubes and 20 µl proteinase K.
4. Incubate at 55 °C overnight with rocking or for several hours with occasional mild vortexing every 15 minutes.
5. Cool to room temperature. Vortex 20 seconds and Centrifuge 12000rpm for 5 minutes.
6. Pipette 300 µl of supernatant into a spin column (if pellet not visible, repeat previous step) and add 300 µl AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes.
7. Centrifuge 4000 rpm for 2 minutes and discard the flow-through.
8. Add 500 µl of Wash Solution, and spin at 8,000 rpm for 1 minute.
9. Repeat washing step 8
10. Discard flow-through. Spin at 10,000 rpm for an additional minute to remove residual amount of Wash Solution.
11. Place the column into a clean 1.5 ml microfuge tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate the tube at 37 or 50 °C for 2 minutes. Incubation at 37 or 50 °C could increase recovery yield.
12. Spin at 10,000 rpm for 1 minute to elute DNA from the column.
13. Measure DNA quantity by UV absorption at A₂₆₀ (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel. Isolated genomic DNA should not contain RNA. Its length should be over 50 kb.

For Cultured Animal Cell

1. Centrifuge the appropriate number of cells(>5x10⁶) for 5 minutes at 1200 rpm.
2. Resuspend pellet in 500 µl of PBS Solution, and wash cells two times with PBS.
3. Resuspend pellet in 300 µl of ACL Solution Buffer
4. Add 20µl of Proteinase K .
5. Incubate at 55 °C for 10 minutes.
6. Cool to room temperature. Vortex for 20 seconds and Centrifuge 12000 rpm for 5 minutes.
7. Pipette 200 µl of supernatant into a spin column (if pellet not visible, repeat previous step) and add 200 µl AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes.
8. Centrifuge 4000 rpm for 2 minutes and discard the flow-through.
9. Add 500 µl of Wash Solution, and spin at 8,000 rpm for 1 minute.
10. Repeat washing step 9
11. Discard flow-through. Spin at 10,000 rpm for an additional minute to remove residual amount of Wash Solution.
12. Place the column into a clean 1.5 ml microfuge tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate the tube at room temperature for 2 minutes. Incubation at 37 or 50 °C could increase recovery yield.
13. Spin at 10,000 rpm for 1 minute to elute DNA from the column.
14. Measure DNA quantity by UV absorption at A₂₆₀ (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel. Isolated genomic DNA should not contain RNA. Its length should be over 50 kb.

Use of IDPURE™ Spin Column PCR Products Purification Kit does not guarantee the successful outcome of any molecular analysis.