Tribioscience

Column-Pure Animal Genomic DNA Isolation Kit

Catalog	Unit
TBS6102	50 Preparations

Description

Column-Pure Animal Genomic DNA Isolation Kit is a quick and easy spin column method for the isolation of genomic DNA from various animal tissues or cells. No phenol, chloroform, or other hazardous organic solutions are required. Proteins are degraded by Proteinase K, DNA is bound to the silica-based filter in the spin column, impurities are washed away, and the purified gDNA is eluted from the binding matrix of the column. The isolated genomic DNA is of excellent quality for PCR, restriction digestion, and other downstream applications. The kit contains all necessary tubes for the complete experiment in isolation of genomic DNA.

Component

Product Component	Quantity
ACL Solution[a]	20 ml
PBS Solution	75 ml
AB Solution	20 ml
Proteinase K[b]	20 mg
Wash Solution[c]	12 ml
Elution Buffer[d]	5 ml
Column-Pure Spin Column (with 2.0-ml Collection Tube)	50
Size	50 Preps

a. ACL Solution may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution to 37ºC.
b. Before use, add 1 ml of sterilized water to the tube containing 20 mg of Proteinase K. Keep solution at -20ºC.
c. Before use, add 48 ml of 100% ethanol to 12 ml Wash Solution.
d. 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.

Store at 4°C. Store Proteinase K at -20°C

Protocol for Animal Tissue

1. Cut up to 30 mg of tissue and place in a 1.5 ml centrifuge tube.
2. Add 300 µl of ACL Solution (Animal Cell Lysis Solution) to 1.5 ml centrifuge tube and 20 µl of Proteinase K.
3. Incubate at 55ºC until tissues are completely lysed (usually 1-3 hours). Vortex occasionally. Incubating the sample in a shaking water bath can reduce lysis time.
   • If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 minutes at room temperature before continuing to step 5.
4. Cool to room temperature. Vortex for 20 seconds and centrifuge 10,000 x g (12,000 rpm) for 5 minutes.
5. Pipette 300 µl of supernatant to a new Eppendorf tube, add 300 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution into a Column-Pure Spin Column.
6. Centrifuge at 2,000 x g (4,000 rpm) for 2 minutes and discard the flow-through.
7. Add 500 µl of Wash Solution, and spin at 8,000 x g (10,000 rpm) for 2 minutes. Discard the flow-through.
8. Repeat Step 7.
9. Spin at 8,000 x g (10,000 rpm) for an additional minute to remove residual amounts of Wash Solution.
10. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of the membrane in the column. Incubate at room temperature for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.
11. Spin at 8,000 x g (10,000 rpm) for 1 minute to elute DNA from the column.
12. For long term storage, keep aliquots of purified genomic DNA at -20ºC.
13. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 ug). Assess genomic DNA quality by an analytical 0.7% agarose gel

Protocol for Rodent Tail

1. Place 1.5 ml centrifuge tubes on dry ice.
2. Cut 0.5 cm to 1 cm from ends of tails and place in tubes.
3. Add 300 µl of ACL Solution to 1.5 ml centrifuge tubes and then add 20 µl of Proteinase K.
4. Incubate at 55ºC overnight with rocking; or for several hours with occasional mild vortexing every 15 minutes.
   • If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 minutes at room temperature before continuing to step 6.
5. Cool to room temperature. Vortex for 20 seconds and centrifuge at 10,000 x g (12,000 rpm) for 5 minutes.

6. Pipette 300 µl of supernatant into to a new Eppendorf tube, add 300 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution to a Column-Pure Spin Column.
7. Centrifuge 2,000 x g (4,000 rpm) for 2 minutes and discard the flow-through.
8. Add 500 µl of Wash Solution, and spin at 8,000 x g (10,000 rpm) for 1 minute. Discard the flow-through.
9. Repeat step 8.
10. Spin at 8,000 x g (10,000 rpm) for an additional minute to remove residual amounts of Wash Solution.
11. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of the membrane in the column. Incubate at room temperature for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.
12. Spin at 8,000 x g (10,000 rpm) for 1 minute to elute DNA from the column.
13. For long term storage, keep aliquots of purified genomic DNA at -20ºC.
14. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 µg).
14. Assess genomic DNA quality by an analytical 0.7% agarose gel.

For Cultured Animal Cells

1. Centrifuge the appropriate number of cells (>5x10^6) for 5 minutes at 200 x g (1,200 rpm) and discard the supernatant.
2. Resuspend pellet in 500 µl of PBS Solution, centrifuge for 5 minutes at 200 x g (1,200 rpm) and discard the supernatant.
3. Wash the cells twice with PBS Solution by repeating step 2.
4. Resuspend pellet in 300 µl of ACL solution buffer.
5. Add 20 µl of Proteinase K, mix well and Incubate at 55ºC for 10 minutes.
   • If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 minutes at room temperature before continuing to step 7.
6. Cool to room temperature. Vortex for 20 seconds and centrifuge 10,000 x g (12,000 rpm) for 5 minutes.
7. Pipette 200 µl of supernatant to a new Eppendorf tube, add 200 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution to a Column-Pure Spin Column.
8. Centrifuge at 2,000 x g (4,000 rpm) for 2 minutes and discard the flow-through.
9. Add 500 µl of Wash Solution, and spin at 8,000 x g (10,000 rpm) for 1 minute, discard the flow-through.
10. Repeat step 9.
11. Spin at 8,000 x g (10,000 rpm) for an additional minute to remove residual amounts of Wash Solution.
12. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of the membrane in the column. Incubate at room temperature for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.
13. Spin at 8,000 x g (10,000 rpm) for 2 minutes to elute DNA from the column.
14. For long term storage, keep aliquots of purified genomic DNA at -20ºC.
15. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 ug). Assess genomic DNA quality by an analytical 0.7% agarose gel.

From Paraffin Tissue

1. Excise 25~30 mg paraffin tissue with a clean, sharp scalpel and transfer to a 1.5 ml Eppendorf tube.
2. Add 1.2 ml xylene (not included in the kit) to the tube, then vortex for 3 minutes. Xylene is used to remove paraffin.
3. Centrifuge at 10,000 x g (12,000 rpm) for 5 minutes at room temperature.
4. Discard the supernatant and keep the pellet.
5. Add 1.2 ml 100% of ethanol to the tube. Gently vortex for 1 minute. Incubate at room temperature for 1 minute.
6. Centrifuge at 10,000 x g (12,000 rpm) for 5 minutes at room temperature. Discard supernatant.
7. Repeat steps 4 to 6.
8. Incubate at 37ºC for 10-15 minutes to remove residual ethanol.
9. Resuspend the sample in 200 µl TE buffer, and proceed immediately to step 10.
10. Add 300 µl of ACL Solution (Animal Cell Lysis Solution) and then add 20 µl of Proteinase K.
    • If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 minutes at room temperature before continuing to step 12.
11. Incubate at 55ºC until the tissue is completely lysed (usually 1-3 hours). Vortex occasionally. Incubation in shaking water bath can reduce lysis time.
12. Cool to room temperature. Vortex for 20 seconds and centrifuge at 10,000 x g (12,000 rpm) for 5 minutes.
13. Pipette 300 µl of supernatant to a new Eppendorf tube, add 300 µl of AB Solution. Mix by

occasionally inverting tube, and keep for 2 minutes. Then load all the solution to a Column-Pure Spin Column.

14. Centrifuge at 2,000 x g (4,000 rpm) for 2 minutes and discard the flow-through.
15. Add 500 µl of Wash Solution, and spin at 6,000 x g (8,000 rpm) for 1 minute. Discard the flow-through.
16. Repeat step 15.
17. Spin at 8,000 x g (10,000 rpm) for an additional minute to remove residual amounts of Wash Solution.
18. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of the membrane in the column. Incubate at room temperature for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.
19. Spin at 8,000 x g (10,000 rpm) for 1 minute to elute DNA from the column.
20. For long term storage, keep aliquots of purified genomic DNA at -20ºC.
21. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel.

For research use only