LAMDA
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Column-Pure™ Animal Genomic DNA Kit

Cat. No. D427-100

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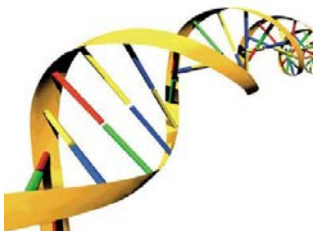

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Catalog No.: D427-100

Product Name: **Column-Pure™ Animal Genomic DNA Kit**

Size: **100 preps**

Description: This is a quick and easy spin column method for isolation of genomic DNA from various animal tissues and cultured cells. The kit is designed to process tissue samples up to 30mg. The isolated DNA is of high quality for many molecular biology applications, such as; PCR, restriction digestion, and other downstream applications.

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Kit Contents:

ACL Solution	2x20ml	Wash Solution	2x12ml
PBS Solution	2x30ml	Elution Buffer	2x5ml
AB Solution	2x20ml	Spin Column	2x50
Proteinase K	2x20mg		

Storage: Store all Solutions/Buffers at room temperature; keep Proteinase K at -20°C.

Caution: Do not inhale or swallow.
Keep away from food, drink, and animal feed.
Keep out of children's reach.
In case of accidental exposure, seek immediate medical attention.

All MSDS are available on request.

Protocol

Procedure for Isolation of Genomic DNA from Animal Tissue

1. Before using the kit, please do the following:

   A. Note that the ACL Solution may form a precipitate upon storage; if necessary, dissolve the precipitate by warming the solution at 37°C.
   B. Add 1ml sterilized water to the tube containing 20mg Proteinase K to dissolve the Proteinase K. Keep solution at -20ºC from now on for long term storage.
   C. Add 48ml of 100% ethanol to the 12ml Wash Solution and mix well.

2. Cut up to 1cm of mouse tail or up to 30mg of tissue and place in a 1.5ml centrifuge tube.
3. Add 300µl of ACL Solution (Animal Cell Lysis Solution) to the centrifuge tube.

4. To lyse the tissue, add 20µl Proteinase K solution and Incubate at 55°C until the tissue is completely lysed.

   Note: a. Mince the tissue to speed-up the lysis process.
   b. Different tissues may need different lengths of incubation time. Generally, it may take 1-5 hours or longer. Overnight incubation; such as, for mouse tail, will not damage the DNA. Vortexing occasionally or incubating in a shaking water bath can reduce the lysis time.

5. When the lysis completes, cool the tube to room temperature, vortex for 20 seconds and centrifuge in a microcentrifuge for 5 minutes at full speed.
6. Pipette 250µl of supernatant into a Spin Column and add 250µl of AB Solution. Mix by inverting the column 4-6 times, and then centrifuge in a microcentrifuge for 2 minutes and discard the flow-through.
7. Add 500µl of Wash Solution; centrifuge for 2 minutes, and discard the flow-through. Repeat this step one more time.
8. Centrifuge the tube for an additional minute to remove any residual Wash Solution.
9. Place the column into a clean 1.5ml centrifuge tube. Add 50-100µl Elution Buffer to the center of the membrane in the column. Incubate the tube at room temperature for 5 minutes. (**Note:** Incubating the tube at 37 or 50ºC, or doing the elution twice may increase DNA yield.)
10. Centrifuge in a microcentrifuge for 1 minute to elute DNA from the column.

Procedure for Isolation of Genomic DNA from Cultured Animal Cells

1. Collect the appropriate number of cells (*about* $5x10^6$) in a 1.5ml microcentrifuge tube.
2. Rinse and wash the cells with 500µl PBS Solution.
3. Resuspend pellet in 300µl of ACL solution.
4. To lyse the cells, add 20µl of Proteinase K and Incubate at 55°C for 10 minutes.
5. When the lysis completes, cool the tube to room temperature, vortex for 20 seconds and centrifuge in a microcentrifuge for 5 minutes at full speed.
6. Pipette 250µl of supernatant into a Spin Column and add 250µl of AB Solution. Mix by inverting the column 4-6 times; then, centrifuge in a microcentrifuge for 2 minutes and discard the flow-through.
7. Add 500µl of Wash Solution, centrifuge for 2 minutes, and discard the flow-through. Repeat this step one more time.
8. Centrifuge the tube for an additional minute to remove any residual Wash Solution.
9. Place the column into a clean 1.5ml centrifuge tube. Add 50-100µl Elution Buffer to the center of the membrane in the column. Incubate the tube at room temperature for 5 minutes. (**Note:** Incubating the tube at 37 or 50ºC, or doing the elution twice may increase DNA yield.)
10. Centrifuge in a microcentrifuge for 1 minute to elute DNA from the column.

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Procedures for Isolation of Genomic DNA from Paraffin Tissue

1. Excise about 30mg paraffin tissue with a clean sharp scalpel, and transfer to a 1.5ml microcentrifuge tube.
2. Add 1.2ml xylene (not included in the kit) to the tube, then vortex for 3 minutes to remove paraffin.
3. Centrifuge in a microcentrifuge for 5 minutes at room temperature.
4. Remove the supernatant completely. Keep the pellet.
5. Add 1.2ml 100% of ethanol to the tube. Gently vortex for 1 minute. Incubate at room temperature for 1 minute. Centrifuge for 5 minutes at room temperature. Discard supernatant completely. Repeat this step one more time
6. Incubate the tube at 37°C for 10-15 minutes to remove residual ethanol.
7. Add 300µl of ACL Solution to the centrifuge tube.
8. To lyse the tissue, add 20µl Proteinase K solution and Incubate at 55°C until the tissue is completely lysed.

   Note: Different tissues may need different lengths of incubation time. Generally, it may take 1-5 hours or longer. Overnight incubation; such as for mouse tail, will not damage the DNA. Vortexing occasionally or incubating in a shaking water bath can reduce the lysis time.

9. When the lysis completes, cool the tube to room temperature, vortex for 20 seconds and centrifuge in a microcentrifuge for 5 minutes.
10. Pipette 250µl of supernatant into a Spin Column and add 250µl of AB Solution. Mix by inverting the column 4-6 times; then, centrifuge in a microcentrifuge for 2 minutes and discard the flow-through.
11. Add 500µl of Wash Solution, centrifuge for 2 minutes, and discard the flow-through. Repeat this step one more time.
12. Centrifuge the tube for an additional minute to remove any residual Wash Solution.
13. Place the column into a clean 1.5ml centrifuge tube. Add 50-100µl Elution Buffer to the center of the membrane in the column. Incubate the tube at room temperature for 5 minutes. (**Note:** Incubating the tube at 37 or 50ºC, or doing the elution twice may increase DNA yield.)
14. Centrifuge in a microcentrifuge for 1 minute to elute DNA from the column.