
LaboPass™ FFPE Mini

Introduction

LaboPass™ FFPE Kit provides a simple and fast method for the isolation of total DNA from up to FFPE tissue section, 40 mg of animal tissue and 5×10^6 cultured cells. The purified DNA using this kit is ready for applications such as PCR, Southern blotting, and restriction enzyme assay.

Storage Conditions

All components of LaboPass™ FFPE Kit, except Proteinase K solution, should be stored in room temperature (15-25°C). Proteinase K solution (20 mg/ml) can be stored for 2 months at 4°C without any decrease in activity. But storage at -20°C with usable aliquots is recommended for prolonged preservation. Under cool ambient conditions, a precipitate may form in buffer TB. In such a case, heat the bottle at 60°C to dissolve. LaboPass™ FFPE Kit is guaranteed for 1 year after delivered to an end user.

Kit Contents

Cat. No.	CMBA0111	CMBA0112	CMBA0115
No. of preparation	50	200	1000
Spin column	50	200	1000
Collection tube	200	800	4000
Buffer TL	15ml	60ml	60ml x5
Buffer TB	24ml	100ml	100ml x5
Buffer BW (conc.)	19.6ml x2	76.1ml x2	76.1ml x10
Buffer NW (conc.)	10.5ml	32.3ml	32.3ml x5
Buffer AE	15ml	60ml	60ml x5
Proteinase K solution (20 mg/ml) ⁽¹⁾	1.1ml	1.1ml x4	1.1ml x20

(1) Avoid repeated freezing and thawing. Once thawed, store at 4°C for a long time.

* All Solution (Buffer) is available to purchase separately.

(2) Does not contains Xylene and 96~100% Ethanol

Little copurification with RNA

LaboPass™ FFPE spin column has very stronger affinity to DNA than RNA. Although RNase is not treated, the percentage of RNA from total prepared nucleic acid will be very low even with a transcriptionally active tissue, such as liver or pancreas.

However, if RNA-free DNA is required, RNase should be treated.

Protocols for FFPE Tissue section

Before experiment

- *Prepare the water bath or Heat Block to 56°C and 90°C*
- *Equilibrate buffer AE to room temperature*
- *Add appropriate volume of absolute ethanol to Buffer BW and NW before first use.*
- *All centrifugation should be performed at room temperature.*
- *Mix Buffer TL and TB thoroughly by shaking before use.*
- *Buffer TB may precipitate at cool ambient temperature. If so, dissolve it in 60°C water bath.*

1. Using a scalpel, trim excess paraffin off the sample block.

2. Cut up to 8 sections 5–10 µm thick.

If the sample surface has been exposed to air, discard the first 2–3 sections.

3. Immediately place the sections in a 1.5 or 2 ml microcentrifuge tube (not supplied), and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 10 s.

4. Centrifuge at full speed for 2 min at room temperature (15–25°C).

5. Remove the supernatant by pipetting. Do not remove any of the pellet.

6. Add 1 ml ethanol (96–100%) to the pellet, and mix by vortexing.

The ethanol extracts residual xylene from the sample.

7. Centrifuge at full speed for 2 min at room temperature.

8. Remove the supernatant by pipetting. Do not remove any of the pellet.

Carefully remove any residual ethanol using a fine pipet tip.

9. Open the tube and incubate at room temperature or up to 37°C. Incubate for 10 min or until all residual ethanol has evaporated.

10. Add 200 μl of TL buffer, 20 μl of Proteinase K solution and mix by vortex. Incubate the lysate for 1 h at 56°C.

Longer incubation will not affect DNA recovery.

12. Incubate at 90°C for 1 h.

The incubation at 90°C in Buffer TL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 90°C.

13. Check!! If Buffer TB precipitates, pre-heat in a 60°C water bath to dissolve completely.

14. Spin down the tube briefly to remove any drops from inside of the lid.

15. Optional: If RNA-free DNA is required, add 4 μl of RNase solution (100 mg/ml, not provided), vortex to mix thoroughly, and incubate for 2 min at room temperature.

LaboPass™ FFPE spin column has the very stronger affinity to DNA than RNA. Unless RNase is treated, RNA occupies very small portion of eluates. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

16. Add 400 $\mu\ell$ of Buffer TB. Immediately vortex the tube to mix thoroughly. Spin down the tube briefly to remove any drops from inside of the lid.

17. Apply the mixture to the spin column. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Replace the collection tube with new one. (provided)

If more than 700 $\mu\ell$ of mixture volume is processed, apply the mixture twice; apply 700 $\mu\ell$ of the mixture, spin down, discard the flow-through, reinsert empty collection tube, and repeat the steps again until all of the mixture is applied to the spin column. If the mixture has not passed completely through the membrane, centrifuge again at full speed until all of the solution has passed through. Centrifuge at maximum speed will not affect the DNA recovery.

18. Add 700 $\mu\ell$ of Buffer BW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Replace the collection tube with new one. (provided)

There is no need to increase the volume of Buffer BW and NW for larger sample.

19. Repeat step 18, Add 700 $\mu\ell$ of Buffer BW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Replace the collection tube with new one. (provided)

20. Add 500 $\mu\ell$ of Buffer NW. Centrifuge for 3 min at 14,000 x g (full speed). Place the spin column in a clean 1.5 ml tube. (not provided)

Care must be taken at this step for eliminating the carryover of buffer NW. If carryover of buffer NW occurs, centrifuge again for 1 min at full speed with the empty collection tube (not provided) before transferring to the new 1.5 ml tube.

21. Add 200 $\mu\ell$ of Buffer AE or distilled water. Incubate for 2 min at room temperature. Centrifuge at 6,000 x g above (>8,000 rpm) for 1 min.

Ensure that the buffer AE or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. If higher concentration of eluate is needed or starting amount of sample is very small, elution volume can be decreased to 50 $\mu\ell$ minimum. However, the small volume of elution buffer will decrease the total amount of DNA recovery. For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water or Tris-Cl (>pH8.5). When using water for elution, check the pH of water (>pH7.0) before elution.

Protocols for Animal Tissue

Before experiment

- *Prepare the water bath to 56°C*
- *Equilibrate buffer AE to room temperature*
- *Add appropriate volume of absolute ethanol to Buffer BW and NW before first use.*
- *All centrifugation should be performed at room temperature.*
- *Mix Buffer TL and TB thoroughly by shaking before use.*
- *Buffer TB may precipitate at cool ambient temperature. If so, dissolve it in 60°C water bath.*

1. Homogenize up to 20 mg of tissue as described in step 1A, 1B, or 1C.

For spleen tissue, up to 10 mg can be processed. If the sample is larger than 20 mg (if spleen, 10 mg), increase the volume of buffer TL proportionally. For 40 mg of liver tissue, 400 μl of buffer TL is required.

1A. For soft tissue, such as liver or brain, put the tissue into 1.5 ml tube, add 200 μl of Buffer TL, and homogenize thoroughly with microhomogenizer.

1B. If microhomogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle. Put the powdered tissue into 1.5 ml tube. Add 200 μl of Buffer TL and pulse-vortex for 15 seconds.

1C. If neither 1A nor 1B is available, mince the tissue with sharp blade as small as possible. Put the tissue into a 1.5 ml tube. Add 200 μl of Buffer TL and pulse-vortex for 15 seconds.

2. Add 20 $\mu\ell$ of Proteinase K solution. Mix by vortexing. Incubate at 56°C until the tissue is completely lysed.

If the sample is larger than 20 mg (if spleen, 10 mg), increase the amount of Proteinase K proportionally. For 40 mg of liver tissue, 40 $\mu\ell$ of Proteinase K solution is required. Lysis time varies from 10 min to 3 hr usually depending on the type of tissue processed and the lysis method used. The lysate will become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.

If the sample is lysed in water bath or heating block, vortex occasionally (2-3 times per hour) during incubation to lysis readily. ***Lysis in shaking water bath, shaking incubator or agitator is best for efficient lysis.***

3. Check!! If Buffer TB precipitates, pre-heat in a 60°C water bath to dissolve completely.

4. Spin down the tube briefly to remove any drops from inside of the lid.

5. Optional: If RNA-free DNA is required, add 4 $\mu\ell$ of RNase solution (100 mg/ml, not provided), vortex to mix thoroughly, and incubate for 2 min at room temperature.

LaboPass™ FFPE spin column has the very stronger affinity to DNA than RNA. Unless RNase is treated, RNA occupies very small portion of eluates. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

6. Add 400 $\mu\ell$ of Buffer TB. Immediately vortex the tube to mix thoroughly. Spin down the tube briefly to remove any drops from inside of the lid

If the sample is larger than 20 mg (if spleen, 10 mg), increase the volume of buffer TB proportionally. For 40 mg of liver tissue, 800 $\mu\ell$ of buffer TB is required.

7. Apply the mixture to the spin column. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Replace the collection tube with new one. (provided)

If more than 20 mg (10 mg spleen) of tissue is processed, apply the mixture twice; apply 700 $\mu\ell$ of the mixture, spin down, discard the flow-through, reinsert empty collection tube, and repeat the steps again until all of the mixture is applied to the spin column. If the mixture has not passed completely through the membrane, centrifuge again at full speed until all of the solution has passed through. Centrifuge at maximum speed will not affect the DNA recovery.

8. Add 700 $\mu\ell$ of Buffer BW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Replace the collection tube with new one. (provided)

There is no need to increase the volume of Buffer BW and NW for larger sample.

9. Add 500 $\mu\ell$ of Buffer NW. Centrifuge for 3 min at 14,000 x g (full speed). Place the spin column in a clean 1.5 ml tube. (not provided)

Care must be taken at this step for eliminating the carryover of buffer NW. If carryover of buffer NW occurs, centrifuge again for 1 min at full speed with the empty collection tube (not provided) before transferring to the new 1.5 ml tube.

10. Add 200 $\mu\ell$ of Buffer AE or distilled water. Incubate for 2 min at room temperature. Centrifuge at 6,000 x g above (>8,000 rpm) for 1 min.

Ensure that the buffer AE or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. If higher concentration of eluate is needed or starting amount of sample is very small, elution volume can be decreased to 50 $\mu\ell$ minimum. However, the small volume of elution buffer will decrease the total amount of DNA recovery. For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water or Tris-Cl (>pH8.5). When using water for elution, check the pH of water (>pH7.0) before elution.

Protocols for Cultured Animal Cells

Before experiment

- *Prepare the water bath to 56°C*
- *Equilibrate buffer AE to room temperature*
- *Add appropriate volume of absolute ethanol to Buffer BW and NW before first use.*
- *All centrifugation should be performed at room temperature.*
- *Buffer TB may precipitate at cool ambient temperature. If so, dissolve it in 60°C water bath.*
- *Mix Buffer TL and TB thoroughly by shaking before use.*

Washing cells with sufficient volume of PBS before procedures usually brings about better results.

1. Harvest cells (up to 5×10^6 cells) to a 1.5 ml microcentrifuge tube by centrifugation at 14,000 x g for 10 sec.

Alternatively, cells can be pelleted at 500 x g for 5 min. For adherent cells, trypsinize the cells before harvesting.

2. Discard the supernatant as much as possible and resuspend cell pellet in 200 μl of Buffer TL.

3. Add 20 μl of Proteinase K solution. Mix by vortexing. Incubate for 10 min at 56°C.

Longer incubation will not affect DNA recovery.

4. Continue with Tissue Protocols from step 3.

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