LaboPass ^{TM} Tissue Mini

Introduction

LaboPass ^{TM} Tissue Kit provides a simple and fast method for the isolation of total DNA from up to 40 mg of animal tissue or 5 x 10^6 cultured cells. The purified DNA using this kit is ready for applications such as PCR, Southern blotting, and restriction enzyme assay. Added **Buffer RL** (**Optional!**) to LaboPass ^{TM} Tissue Kit also provides additional preparation from up to 1 ml of mammalian whole blood.

Storage Conditions

All components of LaboPass ^{TM} Tissue Kit, except Proteinase K solution, should be stored in room temperature(15-25℃). Proteinase K solution (20 mg/ml) can be stored for 2 months at 4℃ without any decrease in activity. But storage at -20℃ 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℃ to dissolve. LaboPass ^{TM} Tissue Kit is guaranteed for 1 year after delivered to an end user.

Sample amount and expected yields

LaboPass ^{TM} Tissue kit is designed for preparation from small amount of tissue. For most animal tissues, it is recommended the starting amount of sample should not be over 20 mg per each prep. If it exceeds about 25-30 mg depending on the tissue type, the efficiency of DNA recovery will be significantly reduced. We recommend you start with smaller sample amount, and depending on the yield obtained, increase the sample size in subsequent preparations.

The yields and recommended maximum weights of starting materials for various sources are listed below.

Kit Contents

Cat. No.	CME0111	CME0112	CME0115
No. of preparation	50	200	1000
Spin column	50	200	1000
Collection tube	150	600	3000
Buffer TL	15ml	60ml	60mlx5
Buffer TB	24ml	100ml	100mlx5
Buffer BW(conc.)	19.6ml	76.1ml	76.1mlx5
Buffer NW(conc.)	10.5ml	32.3ml	32.3mlx5
Buffer AE	15ml	60ml	60mlx5
Proteinase K solution (20mg/ml)	1.1ml	1.1mlx4	1.1mlx20
Optional! Buffer RL[(1)]	60ml	220ml	

(1) Optional! Buffer RL is available to purchase.

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

Little copurification with RNA

LaboPass TM Tissue 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 Animal Tissue

Before experiment

– Prepare the water bath to 56℃

– 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℃ 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, 10mg), increase the volume of buffer TL proportionally. For 40mg of liver tissue, 400㎕ of buffer TL is required.

1A. For soft tissue, such as liver or brain, put the tissue into 1.5 ml tube, add 200 ㎕ 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 ㎕ 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 ㎕ of Buffer TL and pulse-vortex for 15 seconds.

2. Add 20 ㎕ of Proteinase K solution. Mix by vortexing. Incubate at 56℃ 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 ㎕ 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℃ 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㎕ of RNase solution (100mg/ml, not provided), vortex to mix thoroughly, and incubate for 2 min at room temperature.

LaboPass TM Tissue 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㎕ 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, 800μℓ of buffer TB is required.

7. Apply the mixture to the spin column. Centrifuge for 1 min at 6,000 xg 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 700μℓ 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 μℓ of Buffer BW. Centrifuge for 1 min at 6,000 xg 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 500μℓ of Buffer NW. Centrifuge for 3 min at 14,000 xg (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 200μℓ of Buffer AE or distilled water. Incubate for 2 min

at room temperature. Centrifuge at 6,000 xg 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℃

- 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℃ 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 $5x10^6$ cells) to a 1.5 ml microcentrifuge tube by centrifugation at 14,000 xg for 10 sec.

Alternatively, cells can be pelleted at 500 xg 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 ㎕ of Buffer TL.

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

Longer incubation will not affect DNA recovery.

4. Continue with Tissue Protocols from step 3.

Protocols for Mammalian whole blood 300 ㎕ (*Optional! It needs Buffer RL*)

Before experiment

- Prepare the water bath to 56℃

- 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℃ water bath.

1. Transfer 300 ㎕ of whole blood to a new 1.5 ml tube.

Before transfer of blood, it is recommended that gently rocking the tube of blood until thoroughly mixed.

2. Add 900 ㎕ of Buffer RL to the tube containing the blood sample. Invert the tube 5-6 times to mix. Incubate the mixture for 10 min at room temperature.

Invert 2-3 times during the incubation. The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or mis-stored sample, and you should read the annotation of step 3. If fresh or well-stored sample is processed, it will take less times than 10 min to acquire translucent mixtures. This kit can process up to 1 ml of whole blood sample. If the sample is larger than 300 ㎕, see the detailed protocol for larger sample volume.

3. Centrifuge for 1 min at 14,000 xg (full speed) and carefully

remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet by vortexing or flickering.

Approximately 10-20 ㎕ of residual liquid will remain. Steps 2-3 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white (or pink) pellet before processing next steps. If blood sample has been frozen or mis-stored, resuspend the pellet and repeat step 2-3 until lysate become translucent.

4. Check!! If Buffer TB precipitates, pre-heat in a 60℃ water bath to dissolve Completely.

5. Add 200 ㎕ of Buffer TL to the tube containing the resuspended cells. Pipet the solution 5-6 times to mix well.

The lysate may be viscous.

6. Add 10 ㎕ of Proteinase K solution. Mix by vortexing. Incubate for 10 min at 56 ℃.

Overnight lysis is available and it will not influence the preparation.

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

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

LaboPass TM Tissue spin column has the very stronger affinity to DNA than RNA. Unless RNase is treated, RNA will occupy very small portion of

elutes. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

9. Add 400 ㎕ 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 Buffer TB precipitates, pre-heat in a 60℃ water bath to dissolve completely.

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

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 full speed will not affect the DNA recovery.

11. Add 700 ㎕ of Buffer BW. Centrifuge for 1 min. at 6,000 xg above (>8,000 rpm). Replace the collection tube with new one (provided).

12. Add 500 ㎕ of Buffer NW. Centrifuge for 3 min at 14,000 xg (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.

13. Add 200 ㎕ of Buffer AE or distilled water. Incubate for 2

min at room temperature. Centrifuge at 6,000 xg above (>8,000 rpm).

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 volume of blood sample is very low, elution volume can be decreased to 50 ㎕ 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 Mammalian whole blood 1 ml

(*Optional! It needs Buffer RL.*)

Before experiment

- Prepare the water bath to 65℃

- 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℃ water bath.

1. Transfer up to 1 ml of whole blood to a new 15 ml tube.

Before transfer of blood, it is recommended that gently rocking the tube of blood until thoroughly mixed.

2. Add 3 ml of Buffer RL to the tube containing the blood sample. Invert the tube 5-6 times to mix. Incubate the mixture for 10 min at room temperature.

Invert 2-3 times during the incubation. The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or mis-stored sample, and you should read the annotation of step 3. If fresh or well-stored sample is processed, it will take less time than 10 minutes to acquire translucent mixtures.

3. Centrifuge for 10 min at 2,000 xg (3,000 rpm) and carefully remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet by vortexing or flickering.

Approximately 30-40 ㎕ of residual liquid will remain. Steps 2-3 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white (or pink) pellet before processing next steps. If blood sample has been frozen or mis-stored, resuspend the pellet and repeat step 2-3 until lysate become translucent.

4. Check!! If Buffer TB precipitates, pre-heat in a 60 ℃ water bath to dissolve completely.

5. Add 200 ㎕ of Buffer TL to the tube containing the resuspended cells. Pipet the solution 5-6 times to mix well. Transfer the lysate to a new 1.5 ml tube.

The lysate may be viscous.

6. Add 20 ㎕ of Proteinase K solution. Mix by vortexing. Incubate for 10 min at 64 ℃.

7. Continue with Whole Blood 300 ㎕ protocols from step 7.