LaboPass ™ Blood mini

Protocol Book

LaboPass ™ Blood Mini

Introduction

LaboPass ™ Blood Mini Kit provides a fast and convenient method for the isolation of total DNA from up to 400μLof fresh and frozen mammalian whole blood. It takes about 20 minutes to complete all processing of samples. The purified DNA using this kit is ready for applications such as PCR, Southern blotting, and restriction enzyme assay. The LaboPass ™ Blood Mini Plus Kit also provides additional preparation of animal tissue or other samples.

Storage Conditions

All components of LaboPass ™ Blood Mini 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 BL. In such a case, heat the bottle at 55℃ to dissolve.
LaboPass ™ Blood Mini Kit is guaranteed for 1 year after delivered to an end user.

Kit Contents

Cat. No.	CMB0111	CMB0112	CMB0115
No. of preparation	**50**	**200**	**1000**
Spin column	**50**	**200**	**1000**
Collection tube	**50**	**200**	**1000**
Buffer BL	**12ml**	**50ml**	**50mlx5**
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 AL(1)	**16ml**	**50ml**	

(1) Optional! Buffer AL is available to purchase.
* All Solution (Buffer) is available to purchase separately.

General Information

The yield of purified DNA depends on the number of cells in the sample. 200µL starting blood volume will yield 4 – 12 µg of DNA. Preparation of buffy coat is recommended if higher yield is required. An average of 6µg of total DNA from 200µL of whole human blood, and up to 30µg of DNA from 200µL of buffy coat, or 5 x 10^6 lymphocytes or cultured cells can be purified. DNA and RNA will be copurified unless RNase is treated. RNA may inhibit some downstream enzymatic reactions, but not PCR itself. If RNA-free DNA is required, RNase should be treated before addition of Buffer BL. DNA purified by the LaboPass ™ Blood procedure is free of protein and other contaminants, which may inhibit PCR or other enzymatic reactions. DNA can be used immediately or safety stored in Buffer AE at -20℃ for later use.

Protocols for whole blood and body fluid

Before experiment

- Prepare the water bath to 56℃
- Prepare absolute ethanol
- If a precipitate has formed in buffer BL, heat to dissolve at 60℃.
- 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.

1. Pipette 20µL of Proteinase K solution (20 mg/ml) into the bottom of a 1.5 ml tube.

If the sample volume is larger than 200µL (up to 400µL, increase the amount of Proteinase K proportionally. For 400µL of whole blood, 40µL of Proteinase K solution is needed.

2. Transfer 200µL of whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10^6 lymphocytes in 200µL PBS.

If the sample volume is less than 200µL adjust the volume to 200µL with PBS. If RNA-free DNA is required, add 4µL of RNase (100 mg/ml, not provided) to the sample at this step.

3. Add 200µL of Buffer BL to the tube. Vortex the tube to mix thoroughly.

If the sample volume is larger than 200µL increase the volume of buffer BL in proportion.

4. Incubate at 56℃ for 10 min.

Longer incubation will not affect DNA recovery.

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

6. Add 200µL of absolute ethanol (not provided) to the sample, Pulsevortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.

If the sample volume is larger than 200µL increase the buffer BL volume proportionally.

7. Transfer the mixture to the spin column carefully, centrifuge for 1 min at 6,000 xg above (>8,000 rpm), and replace the collection tube with new one. (provided)

If more than 200µL of sample is processed, apply the mixture twice; apply 700 µL of the mixture, spin down, discard the flow-through, re-insert empty collection tube, and repeat the steps again until all of the mixture has 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 700µL of Buffer BW, centrifuge for 1 min. at 6,000 xg above (>8,000 rpm), and replace the collection tube with new one. (provided)

Though the larger sample than 200µL there is no need to increase the volume of Buffer BW and NW.

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 remnants 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 a 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 volume of blood sample is very low, elution volume can be decreased to 50µL –100µL 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 (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, check the pH of water before elution.

Protocols for Cultured Animal Cells

Before experiment

- Prepare the water bath to 56℃
- Prepare absolute ethanol
- If a precipitate has formed in buffer BL, heat to dissolve at 60℃.
- 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.

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

1. Harvest cells (up to 5x10 cells) to a 1.5 ml microcentrifuge tube and spin down at 14,000 xg for 10 seconds.

Alternatively, cells can be pelleted at 500 xg for 5 minutes. For adherent cells, trypsinize the cells before harvesting.

2. Discard supernatant and resuspend cell pellet in PBS to a final volume of 200µL

3. Add 20µL of Proteinase K solution and vortex briefly.

4. Add 200µL of Buffer BL to the tube. Vortex the tube to mix thoroughly.

If the sample volume is larger than 200µL increase the volume of buffer BL in proportion.

5. Incubate at 56℃ for 10 min.

Longer incubation will not affect DNA recovery.

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

7. Continue with Blood protocols from step 5.

Protocols for Animal Tissue (Optional! It needs the Buffer RL.)

Before experiment

- Prepare the 56℃ and 70℃ water bath
- Prepare absolute ethanol
- If a precipitate has formed in buffer BL, heat to dissolve at 60℃.
- 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

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 per prep. If the sample is larger than 20 mg (if spleen, 10 mg), increase the volume of buffer AL proportionally. For 40 mg of liver tissue, 360µL of buffer AL is required.

1A. For soft tissue, such as liver or brain, put the tissue into 1.5 ml tube, add 180µL of Buffer AL, 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 180µL of Buffer AL 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 180µL of Buffer AL and pulse-vortex for 15 seconds.

2. Add 20µL of Proteinase K solution. Mix by vortexing. Incubate at 56℃ until the tissue is completely lysed.

If the sample is larger than 20 mg, increase the amount of Proteinase K in proportion. For 40 mg of liver tissue, 40µL of Proteinase K solution is required. Lysis time varies from 10 min to 3 hours depending on the type of tissue processed and the lysis method used. After complete lysis, the mixture should become semitransparent without any particles. 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. Spin down briefly to remove any drops from inside of the lid.

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

5. Add 200µL of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 70 ℃ for 10 min.

If the sample is larger than 20 mg, increase the volume of buffer BL in proportion. Longer incubation will not affect DNA recovery.

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

7. Add 200µL of absolute ethanol (not provided) to the sample, Pulsevortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.

If the sample is larger than 20 mg, increase the volume of ethanol proportionally.

8. Transfer the mixture to the spin column carefully, centrifuge for 1 min. at 6,000 xg above (>8,000 rpm), and replace the collection tube with new one. (provided)

If more than 20 mg of sample is processed, apply the mixture twice; apply 700 µL of the mixture, spin down, discard the flow-through, re-insert empty collection tube, and repeat the steps again until all of the mixture has 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.

9. Add 700µL of Buffer BW, centrifuge for 1 min. at 6,000 xg above (>8,000 rpm), and replace the collection tube with new one. (provided)

Though the larger sample than 20 mg, there is no need to increase the volume of Buffer BW and NW.

10. 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 remnants 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 a new 1.5 ml tube.

11. Add 200µL 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 amount of sample is very small, elution volume can be decreased to 50µL –100µL 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 (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, check the pH of water before elution.

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