UE Blood Genomic DNA Miniprep Kit

This kit is based on the efficient release of genomic DNA from anti-coagulated whole blood by a special cell lysis and heme/protein precipitation buffer coupled with the selective adsorption of the genomic DNA to a special column. The protocol is suitable for purifying up to 12 μg of genomic DNA from 250 μl of anticoagulated whole blood.

1. Kit contents, storage and stability

Cat. No.	UE-MN-BL-GDNA-10	UE-MN-BL-GDNA-50	UE-MN-BL-GDNA-250
Kit size	**10 preps**	**50 preps**	**250 preps**
Miniprep column	**10**	**50**	**250**
2 ml microfuge tube	**10**	**50**	**250**
1.5 ml microfuge tube	**10**	**50**	**250**
Buffer AP1	**6 ml**	**30 ml**	**150 ml**
Buffer AP2	**2 ml**	**6 ml**	**30 ml**
Buffer W1A concentrate	**5 ml**	**24 ml**	**120 ml**
Buffer W2 concentrate	**5 ml**	**24 ml**	**2×72 ml**
Buffer TE	**3 ml**	**11 ml**	**60 ml**
Protocol manual	**1**	**1**	**1**

Buffer AP1: Cell lysis buffer. Store at room temperature.

Buffer AP2: Protein-depleting buffer. Store at room temperature.

Buffer W1A concentrate: Wash buffer. Store at room temperature. Add amount of ethanol specified on the bottle label and store at room temperature. Either 100% or 95% denatured ethanol can be used.

Buffer W2 concentrate: Desalting buffer. Add amount of ethanol specified and store at room temperature. Either 100% or 95% denatured ethanol can be used.

Buffer TE: 5 mM Tris-HCl, 0.1 mM EDTA, pH 8.5. Store at room temperature.

2. Caution

1. If bird blood or amphibian blood is used, because the red blood cells in the blood are nucleated, the blood volume should not exceed 10 μl, and add PBS solution to dilute the blood sample to 250 μl before following the normal procedure.
2. The column has a maximum binding capacity of approximately 25 μg. If more genomic DNA is required, process 0.5 ml of whole blood in the following manner. To extract DNA from 0.5 ml of blood, divide the sample into 2×250 μl aliquots and prepare extracts by following Steps 1-4 in two

separate Microfuge tubes. Combine the supernatant obtained in step 4 into one column to consolidate the genomic DNA and increase the yield. Elute the purified genomic DNA in 100-200 µl of Buffer TE.

3. Buffer AP1 and Buffer W1A contain chemical irritants. When working with these buffers, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.
4. For maintaining the integrity and reactivity of the genomic DNA, particularly in PCR, the purified genomic DNA should be eluted and stored in low-salt Tris buffer containing 0.1 mM EDTA.

3. Preparation before experiment

1) Before using the kit, add the amount of ethanol specified on the bottle label to the Buffer W1A and Buffer W2 concentrates and mix well. Either 100% or 95% (denatured) ethanol can be used.
2) Prepare tips and centrifuge tubes of nucleic acid-free and nuclease-free.

4. Protocols

1. Add 500 µl of Buffer AP1 to a 1.5 ml microfuge tube.
2. Add 200-250 µl of anti-coagulated whole blood. Close the cap of the Microfuge tube and mix by vortexing at top speed for 10 seconds.

Note: Vortexing is required for complete release of the genomic DNA. Although vortexing will result in limited shearing of the genomic DNA, it will have no effect upon the performance of the genomic DNA in applications which require high molecular DNA.

Note: To extract genomic DNA from clotted or dried blood, place the sample in a mortar and add 200 µl of 20 mM Tris, 10 mM EDTA, pH 8.5. Grind rapidly for 30 seconds to disperse the sample. Add 500 µl of Buffer AP1 pre-heated to 50℃ for 1 min and grind briefly or pipette to dissolve the sample. Transfer the sample to a 1.5 ml Microfuge tube with a transfer pipette or other device. Vortex to further dissolve dried or clotted blood, and after cooling in an ice bath, proceed to step 3.

3. Add 100 µl of Buffer AP2 and mix by vortexing at top speed for 10 seconds.
4. Centrifuge at 12,000×g for 10 minutes at ambient temperature to pellet cellular debris.
5. Place a Miniprep column into a 2 ml Microfuge tube. Pipette the clarified supernatant obtained from step 4 into the Miniprep column. Centrifuge at 12,000×g for 1 minute.

Note: If any liquid remains in the column after centrifugation, extend the centrifuge time or increase the g-force.

6. Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Pipette 700 µl of Buffer W1A into the column and allow to stand at room temperature for 2 minutes. Centrifuge at 12,000×g for 30 seconds.

Note: Be sure that ethanol has been added to the W1A concentrate.

Note: If any liquid remains in the Miniprep column after centrifugation, extend the centrifuge time or increase the g-force.

7. Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Add 800 µl of Buffer W2 to the column and centrifuge at 12,000×g for 1 minute.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

8. Optional Step: Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Add 500 µl of Buffer W2 to the Miniprep column and centrifuge at 12,000×g for 1 minute.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

9. Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Centrifuge at 12,000×g for 1 minute.

10. Place the Miniprep column into a 1.5 ml Microfuge tube (provided). Add 80-200 µl of Buffer TE. Allow to stand at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute to elute the genomic DNA.

Note: Pre-warming Buffer TE at 65℃ will generally improve elution efficiently.

5. Overview