

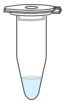
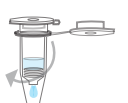


α^+ Solution™
Blood / Cultured Cell Genomic DNA Extraction Mini Kit

Kit Contents:

Cat. No:	APBGR 004 (4 preps_sample)	APBGR 100 (100 preps)	APBGR 200 (200 preps)
RBC Lysis Buffer	7 ml	135 ml	270 ml
TG Buffer	1.5 ml	30 ml	60 ml
BG Buffer	1.5 ml	40 ml	80 ml
BW Buffer	1.5 ml x 2	45 ml	90 ml
Wash Buffer (concentrate) ^a	1 ml	25 ml	35 ml
Elution Buffer	1.5 ml	30 ml	60 ml
BG Mini Column	4 pcs	100 pcs	200 pcs
Collection Tube	8 pcs	200 pcs	400 pcs
User Manual	1	1	1
Preparation of BW Buffer and Wash Buffer by adding ethanol (96 ~ 100%)			
Ethanol volume for Wash Buffer ^a	4 ml	100 ml	140 ml

Brief procedure:

Sample Preparation		Transfer up to 300µl fresh blood to a 1.5ml microcentrifuge tube (not provided).
Lysis		RBC Lysis 1. Add 3 volume of RBC Lysis Buffer and incubate for 10 minutes. 2. Centrifuge at 3,000 x g for 5 minutes. 3. Resuspend the pellet with 100 µl of RBC Lysis Buffer. Cell Lysis 1. Add 200 µl of BG Buffer and incubate for 10 minutes. 2. Add 200µl ethanol (96~100%) .
Binding		Centrifuge at full speed (~10,000 x g) for 1 min.
Wash		1. Add 400 µl of BW Buffer. Centrifuge at full speed (~10,000 x g) for 1 min. 2. Add 600 µl of Wash Buffer. Centrifuge at full speed (~10,000 x g) for 1 min. Repeat this step for one more washing.
Dry		Centrifuge at full speed for an additional 3 min to dry the column.
Elution		Add 100 µl of preheated Elution Buffer or ddH ₂ O (pH 7.5-9.0) Centrifuge at full speed (~18,000 x g) for 2 min.
Pure DNA		

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1. Up to 300 µl of Whole blood 2. Up to 200 µl of frozen blood 3. Up to 200 µl of buffy coat 4. Up to 1 x 10 ⁷ of Cultured animal cells 5. Up to 1 x 10 ⁹ of Cultured bacterial cells
Operation time:	30 minutes
Binding capacity:	up to 60 µg total DNA / column
Expected yield:	4 ~ 8 µg / prep
Column applicability:	centrifugation and vaccum
Minimum elution volume:	50 µl

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. **Add ethanol (96- 100 %) to Wash Buffer when first open.**

General Protocol

Please Read Important Notes Before Starting Following Steps.

STEP	PROCEDURE
1 Sample preparation	1. Collect fresh human blood in an anticoagulant-treat collection tube. 2. Transfer up to 300 µl fresh blood to a 1.5 ml microcentrifuge tube (not provided). If the sample is more than 300 µl (up to 1 ml) , add the sample to a sterile 15 ml centrifuge tube.
2.1 RBC Lysis	1. Add 3 volume of RBC Lysis Buffer and mix by inversion. Do not vortex. 2. Incubate at room temperature for 10 minutes. 3. Centrifuge at 3,000 x g for 5 minutes and completely remove the supernatant. 4. Resuspend the pellet with 100 µl of RBC Lysis Buffer.
2.2 Cell Lysis	1. Add 200 µl of BG Buffer and mix by vortex. 2. Incubate for 10 minutes at room temperature or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. 3. Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70°C water bath. Optional Step: If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.
3 Ethanol Dilution	Add 200 µl ethanol (96-100%) to the sample mixture. Mix immediately and thoroughly by vortexing to yield a homogeneous solution.
4 DNA Binding	Place a BG Mini Column in a Collection Tube. Transfer the mixture (including any precipitate) carefully to the BG Mini Column. Centrifuge at full speed (~10,000 x g) for 1 min then place the BG Mini Column to a new Collection Tube.
5.1 Wash	Add 400µl BW Buffer . Centrifuge for 30 seconds at full speed (10,000 x g) and discard the flow-through.
5.2 Wash	Add 600µl Wash Buffer (ethanol added) . Centrifuge for 30 seconds at full speed (10,000 x g) and discard the flow-through.
6 Dry column	Centrifuge the BG Mini Column at full speed for an additional 3 min to dry the BG Mini Column.

7 DNA Elution	1. Add 100µl of Preheated Elution Buffer or TE to the membrane center of BG Column. Stand BG Column for 3~5 min or until the buffer is absorbed by the membrane. 2. Centrifuge at full speed for 2 min to elute DNA.
Special Protocol : The sample preparation For Buffy Coat	
1 Sample preparation	1. Collect fresh human blood in an anticoagulant-treat collection tube. 2. Transfer up to 300 µl fresh blood to a 1.5 ml microcentrifuge tube (not provided). If the sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube.
2.1 RBC Lysis	1. Transfer up to 200 µl buffy coat to a 1.5 ml microcentrifuge tube (not provided). 2. Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. 3. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 3 minutes. 4. Centrifuge for 1 minutes at full speed (10,000 x g) and completely remove the supernatant. 5. Resuspend the pellet with 500 µl of RBC Lysis Buffer. Then centrifuge for 1 minutes at full speed (10,000 x g) and completely remove the supernatant. 6. Resuspend the pellet with 200 µl of RBC Lysis Buffer. (Mix the tube by vortex only. Be sure the pellet is completely resuspended or the column would be barred when processing binding step.)
2.2 Cell Lysis	1. Add 250 µl of BG Buffer to the sample and mix by vortex. 2. Incubate for 30 minutes at room temperature or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. 3. Preheat required Elution Buffer in a 70℃ water bath. Optional Step : If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.
3 Ethanol Dilution	Add 250 µl ethanol (96-100%) to the sample mixture. Mix immediately and thoroughly by vortexing to yield a homogeneous solution.
4 DNA Binding	Place a BG Mini Column in a Collection Tube. Transfer the mixture (including any precipitate) carefully to the BG Mini Column. Centrifuge at full speed (~10,000 x g) for 1 min then place the BG Mini Column to a new Collection Tube.
5.1 Wash	Add 400µl BW Buffer . Centrifuge for 30 seconds at full speed (10,000 x g) and discard the flow-through.
5.2 Wash	Add 600µl Wash Buffer (ethanol added) . Centrifuge for 30 seconds at full speed (10,000 x g) and discard the flow-through.
6 Dry column	Centrifuge the BG Mini Column at full speed for an additional 3 min to dry the BG Mini Column.
7 DNA Elution	1. Add 100 µl of Preheated Elution Buffer or TE to the membrane center of BG Column. 2. Incubate the BG Column at 37℃ for 10 minutes. 3. Centrifuge for 1 minute at full speed (10,000 x g) to elute the DNA . --HINT : Standard volume for elution is 100 µl. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 µl.

Special Protocol : The sample preparation For Animal Cultured Cells

Additional requirement	1. RNase A (optional) 2. 96~100% ethanol 3. trypsin or cell scraper (for monolayer cell)
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	1. Sample preparation For Cultured Animal Cells: a. Trypsinize the adherent cells before harvesting. b. Transfer the appropriate number of cell (up to 1 x 10⁷) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 6,000 x g for 20 seconds. c. Remove the supernatant and resuspend the cells with 150 µl of RBC Lysis Buffer. For Fresh blood (except human blood): a. The sample volume of mammalian blood (non-nucleated) can be up to 50 µl; the sample volume of nucleated erythrocytes (eg. bird or fish) can be up to 10 µl. b. Add 150 µl of TG Buffer and the blood sample into a 1.5ml microcentrifuge tube (not provided). Mix by vortex. 2. Add 200 µl of BG Buffer to the sample and vortex for 5 seconds. 3. Incubate for 10 minutes at 70℃ or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. 4. Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70℃ water bath. Optional Step : If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature. 5. Follow the General Protocol starting from Step.4 DNA Binding.
Method	

Special Protocol : The sample preparation For Frozen Blood

Additional requirement	1. RNase A (optional) 2. 96~100% ethanol 3. PBS 4. Proteinase K
Method	1. Transfer up to 200 µl sample. --- If the sample volume is less than 200 µl , add the appropriate volume of PBS. 2. Add 30 µl Proteinase K (10 mg/ml) to the sample and briefly mix. Then incubate for 15 minutes at 60℃ . 3. Add 200 µl BG Buffer to the sample and mix by vortex. 4. Incubate in a 70℃ water bath for 15 minutes to lyse the sample. During incubation, invert the sample every 3 minutes. 5. Preheat required Elution Buffer in a 70℃ water bath. Optional Step : If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature. 6. Follow the General Protocol starting from Step 4 DNA Binding.

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