Genomic DNA Isolation Kit

Cat#: orb180520

(Blood/Cultured Cell/Fungus)

Cat No. orb180520

Size: 100 Rxns

Sample: 300 µl of the whole blood

200 µl of the buffy coat

107mammalian cells

5×107 fungus cells

109 bacterial cells

Format: spin column

Column capacity: up to 50 µg

Operation time: within 60 minutes

Description

The Genomic DNA Isolation Kit (Blood/Cultured Cell/Fungus) is designed specifically for genomic DNA isolation from the whole blood, frozen blood, buffy coat, cultured animal/bacterial cells and fungus. This unique buffer system ensures genomic DNA with high yield and good quality from samples. The spin column is designed to purify or concentrate genomic DNA products which have been previously isolated using buffers. The entire procedure can be completed in 1 hour without phenol/ chloroform extraction. Purified genomic DNA is suitable for use in PCR or other enzymatic reactions.

Feature

➢ Delivering high-quality genomic DNA with the fast procedure ➢ Ready-to-use genomic DNA for high performance in any downstream application ➢ Highly purified and high yield genomic DNA can be extracted from various samples ➢ Optimized lysis buffer for the efficient lysis ➢ Designed to rapidly purify high-quality DNA using spin column format

Application

- Southern blotting
- PCR
- SNP genotyping
- Gene cloning

Kit Contents

Contents	orb180520
Buffer CR	100 ml
Buffer CC	35 ml
Buffer CB	45 ml
Buffer W1	45 ml
Buffer W2 (*Add 60 ml of ethanol prior to initial use)	15 ml
Buffer BE	10 ml
Column CC	100 pcs
Collection Tubes	100 pcs

Quality Control

The quality of the Genomic DNA Isolation Kit (Blood/Cultured Cell/Fungus) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Microcentrifuge tubes
- Absolute ethanol
- RNase A (10 mg/ml)

- For the Gram-positive bacteria sample: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0, prepare the lysozyme buffer immediately prior to use)

- For the fungus sample: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM CaCl2; 0.1 M Tris-HCl pH 7.5; 35 mM β- mercaptoethanol)

Protocol

Fresh whole Blood or Buffy Coat

Step 1 Sample Cells Harvesting

1. Collect blood in EDTA-Na2 treated collection tubes (or other anticoagulant mixtures).
2. Transfer up to 300 µl of the blood or 200 µl of the buffy coat to a sterile1.5 ml microcentrifuge tube.
3. Add 900 µl of the Buffer CR and mix by inversion.
4. Incubate the tube at the room temperature for 10 minutes (invert twice during incubation).
5. Centrifuge for 5 minutes at 4,000 x g.
6. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer CR by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of the Buffer CC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes.

   #Pre-heat the Buffer BE to 60°C for Step 6 DNA Elution.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step):

   Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at the room temperature for 5 minutes.

Step 3 Protein Removal

1. Add 400 µl of the Buffer CB to the sample from Step 2 and shake vigorously.
2. Centrifuge at 12,000 x g for 1 minute.(Don`t over 1 minute)

Step 4 DNA Binding

1. Place a Column CC in a 2 ml Collection Tube.

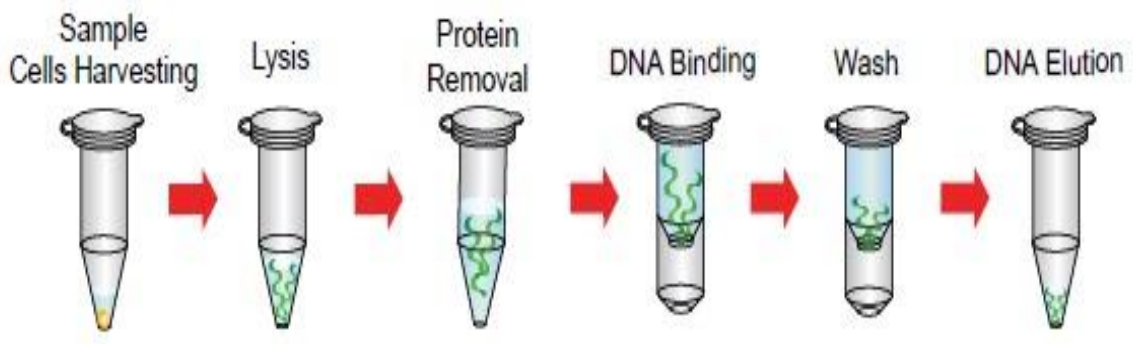
2. Transfer the clear supernatant completely from the previous step to the Column CC.
3. Centrifuge at 14,000 x g for 30 seconds.
4. Discard the flow-through and place the Column CC back in the 2 ml Collection Tube.

Step 5 Wash

1. Add 400 µl of the Buffer W1into the Column CC.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the Column CC back into the same Collection tube.
4. Add 600 µl of the Buffer W2 (Ethanol added) into the Column CC.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the Column CC back into the same Collection tube.
7. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 6 DNA Elution

1. Transfer the dried Column CC to a new 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of the Pre-Heated Buffer BE or TE into the center of the column matrix.
3. Let stand at 60°C for 3 minutes.
4. Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Cultured Mammalian Cells

Step 1 Sample Cells Harvesting

1. Transfer cultured mammalian cells (up to 107) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer CR by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of the Buffer CC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes.

   #Pre-heat the Buffer BE to 60°C for Step 6 DNA Elution.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at the room temperature for 5 minutes.

Step 3 Protein Removal

1. Add 400 µl of the Buffer CB to the sample from Step 2 and shake vigorously.
2. Centrifuge at 12,000 x g for 1 minute.(Don`t over 1 minute)

Step 4 DNA Binding

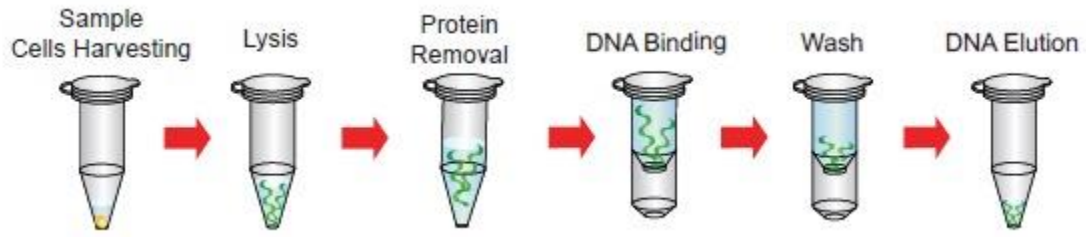
1. Place a Column CC in a 2 ml Collection Tube.
2. Transfer the clear supernatant completely from the previous step to the Column CC.
3. Centrifuge at 14,000 x g for 30 seconds.
4. Discard the flow-through and place the Column CC back in the 2 ml Collection Tube.

Step 5 Wash

1. Add 400 µl of the Buffer W1into the Column CC.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the Column CC back into the same Collection tube.
4. Add 600 µl of the Buffer W2 (Ethanol added) into the Column CC.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the Column CC back into the same Collection tube.
7. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 6 DNA Elution

1. Transfer the dried Column CC to a new 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of the Pre-Heated Buffer BE or TE into the center of the column matrix.
3. Let stand at 60°C for 3 minutes.
4. Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Gram-Negative Bacterial Cells

Step 1 Sample Cells Harvesting

1. Transfer cultured bacterial cells (up to 109) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 50 µl of Buffer CR by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of Buffer CC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes. #Pre-heat the Buffer BE to 60°C for Step 6 DNA Elution.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at the room temperature for 5 minutes.

Step 3 Protein Removal

1. Add 400 µl of the Buffer CB to the sample from Step 2 and shake vigorously.
2. Centrifuge at 12,000 x g for 1 minute.(Don`t over 1 minute)

Step 4 DNA Binding

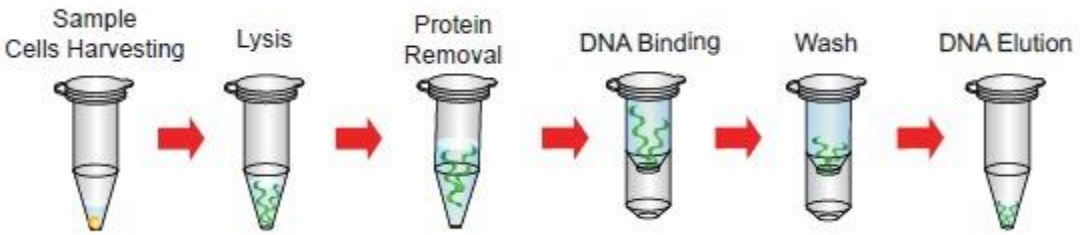
1. Place a Column CC in a 2 ml Collection Tube.
2. Transfer the clear supernatant completely from the previous step to the Column CC.
3. Centrifuge at 14,000 x g for 30 seconds.
4. Discard the flow-through and place the Column CC back in the 2 ml Collection Tube.

Step 5 Wash

1. Add 400 µl of the Buffer W1 into the Column CC.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the Column CC back into the same Collection tube.
4. Add 600 µl of the Buffer W2 (Ethanol added) into the Column CC.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the Column CC back into the same Collection tube.
7. Centrifuge at 14,000 x g again for 2 minutes to remove the residual Buffer W2.

Step 6 DNA Elution

1. Transfer the dried Column CC to a new 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer BE or TE into the center of the column matrix.
3. Let stand at 60°C for 3 minutes.
4. Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Gram-Postive Bacterial Cells

Step 1 Sample Cells Harvesting

1. Transfer cultured bacterial cells (up to 109) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 100 µl of lysozyme buffer by pipetting the pellet. Incubate at room temperature for 20 minutes.

Step 2 Lysis

1. Add 300 µl of Buffer CC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes. #Pre-heat the Buffer BE to 60°C for Step 6 DNA Elution.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 5 minutes.

Step 3 Protein Removal

1. Add 400 µl of Buffer CB to the sample from Step 2 and shake vigorously.
2. Centrifuge at 12,000 x g for 1 minute.(Don`t over 1 minute)

Step 4 DNA Binding

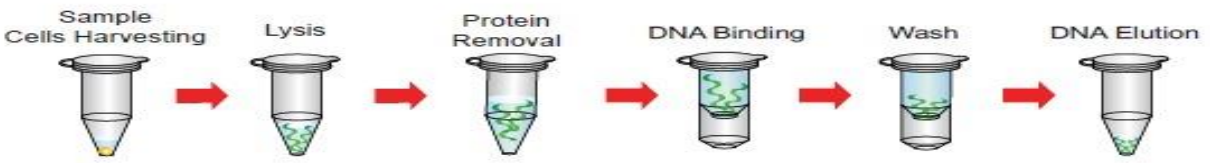
1. Place a Column CC in a 2 ml Collection Tube.
2. Transfer the clear supernatant completely from the previous step to the Column CC.
3. Centrifuge at 14,000 x g for 30 seconds.
4. Discard the flow-through and place the Column CC back in the 2 ml Collection Tube.

Step 5 Wash

1. Add 400 µl of Buffer W1into the Column CC.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the Column CC back into the same Collection tube.
4. Add 600 µl of Buffer W2 (Ethanol added) into the Column CC.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the Column CC back into the same Collection tube.
7. Centrifuge at 14,000 x g again for 2 minutes to remove the residual Buffer W2.

Step 6 DNA Elution

1. Transfer the dried Column CC to a new 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer BE or TE into the center of the column matrix.
3. Let stand at 60°C for 3 minutes.
4. Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying the genomic DNA with the kit.

Problem	Cause	Solution
Low yield of DNA	Incomplete lysed sample	Use the appropriate method for the lysate preparation based on the amount of the starting materials.
		Increase the digestion time.
DNA degrade	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample.
		Use a new sample for the DNA isolation. Perform the extraction of the fresh material when possible.
	Inappropriate sample storage conditions	Store culture cell / fungus at -20°C until use. The whole blood can be stored at 4°C for no longer than 1~2 days.
	DNase contaminant	Use the fresh TAE or TBE electrophoresis buffer.
		Maintain a sterile work environment to avoid contamination from DNases.
Presence of RNA	RNA contamination	Perform RNase A digestion step during Step Lysis.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	Discard the ethanol of the Buffer W2 flow through from the collection tube. Place the spin cartridge into the collection tube and centrifuge the spin cartridge at maximum speed for 2– 3 minutes to completely dry the cartridge.

Caution:

1. During operation, always wear a lab coat, disposable gloves, and protective equipment.
2. Check buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
3. Add 60 ml of the ethanol (96–100%) to the Buffer W2, and shake before use.
4. Buffers W1 contain irritants. Wear gloves when handling these buffers.
5. Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.