Dual Genomic DNA Isolation Kit
(Blood / Cultured Cell / Fungus) (Column Based)
Cat No. PDC02-0100
Size: 100 Reactions

Sample: Up to 300 µl of the whole blood
Up to 200 µl of the frozen blood
Up to 200 µl of the buffy coat
Cultured animal cells (up to 1 x 10^7)
Cultured bacterial cells (up to 1 x 10^9)
Fungus cells (up to 5 x 10^7)

Format: Reagent and spin column
Yield: Up to 50 µg
Operation time: Within 60 minutes
Elution volume: 50~200 µl

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

Kit Contents

Contents	PDC02-0100	PDC02-0100S
Buffer RL	100 ml	4 ml
Buffer CL	35 ml	1.5 ml
Buffer PO	12 ml	0.5 ml
Buffer BD	45 ml	2 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.5 ml x2)
Buffer E	10 ml	1 ml
Column DG	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

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

Required Materials
- 1.5 ml Microcentrifuge tubes
- Isopropanol
- Absolute ethanol (96~100%)
- RNase A (10 mg/ ml) (optional)
- Water bath / Dry bath
- Lysozyme Buffer (for Gram-positive Bacteria):
  20 mg/ml lysozyme, 20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100, pH8.0
- Lyticase or zymolase (for fungus)
- Sorbitol buffer (for fungus):
  1.2M sorbitol, 10mM CaCI2, 0.1M Tris-HCI, pH7.5, 35 mM β-mercaptoethanol

Dual Genomic DNA Isolation Kit Protocol
Fresh whole Blood or Buffy Coat Reagent System Protocol

Step 1 Sample Cells Harvesting
1. Collect blood in the EDTA-Na_2 treated collection tubes (or other anticoagulant mixtures).
2. Transfer up to 300 µl of the blood or 200 µl of buffy coat to a sterile 1.5 ml microcentrifuge tube.
3. Add 900 µl of the Buffer RL and mix by inversion.
4. Incubate the tube at the room temperature for 10 minutes (invert twice during incubation).
5. Centrifuge at 4,000 x g for 5 minutes.
6. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer RL by pipetting the pellet.

Step 2 Lysis
1. Add 300 µl of the Buffer CL 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. During the incubation, invert the tube every 3 minutes.

Optional Step:
◆RNA Degradation (If RNA-free genomic DNA is required, perform this optional step.)
3. 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 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 x g for 3 minutes.
4. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

Switch Step
◆If more pure DNA is required, please switch to **Column System (DNA Pure) Protocol.**

Step 4 DNA Precipitation
1. Add 300 µl of Isopropanol to the sample from the Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 x g for 5 minutes.
3. Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 x g for 3 minutes.
5. Discard the supernatant and air-dry the pellet for 10 minutes.

Step 5 DNA Rehydration
1. Add 50-100 µl of the Buffer E and incubate at 60°C for 5-10 minutes to dissolve the DNA pellet. During the incubation, tap the bottom of the tube to promote DNA rehydration.

Column System (DNA Pure) Protocol

＊Add 60ml of the absolute ethanol to the Buffer W2 prior to initial use.
＊Pre-heat the Buffer E to 60°C prior to use.

Step 1 Sample Preparation

1. Add 400 µl of the Buffer BD to the sample from Step 3 Protein Removal and shake vigorously.

Step 2 DNA Binding

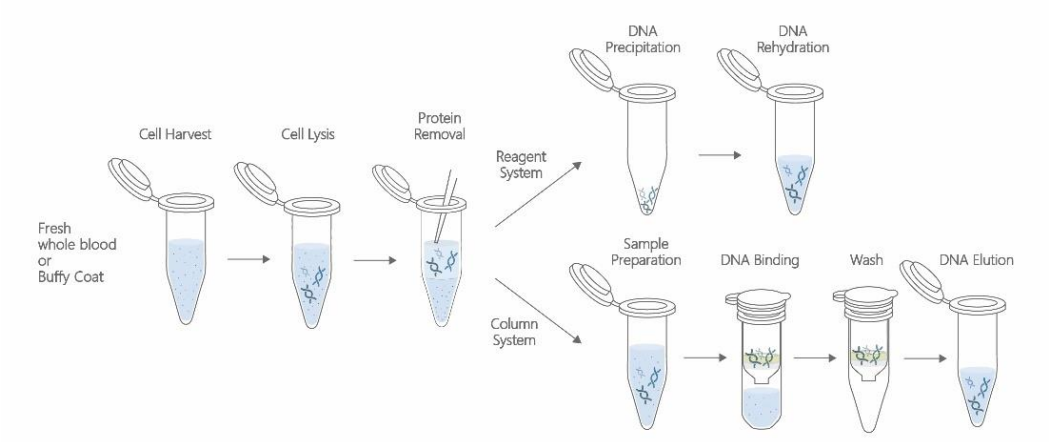
2. Place a Column DG in a 2 ml Collection Tube.
3. Transfer the sample mixture from the previous step to the Column DG.
4. Centrifuge at 14-16,000 x g for 30 seconds.
5. Discard the flow-through and place the Column DG back in the same Collection Tube.

Step 3 Wash

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

Step 4 DNA Elution

1. Place the dried Column DG in a clean 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer E or TE (not provided) into the center of the column matrix.
3. Let it stand at 60°C for 5 minutes.
4. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Dual Genomic DNA Isolation Kit Protocol

Cultured Mammalian Cells Reagent System Protocol

Step 1 Sample Cells Harvesting

1. Transfer cultured mammalian cells (up to 10^7) 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 RL by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of the Buffer CL 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. During the incubation, invert the tube every 3 minutes.

Optional Step:

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

3. 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 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 x g for 3 minutes.
4. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

Switch Step

◆If more pure DNA is required, please switch to **Column System (DNA Pure) Protocol.**

Step 4 DNA Precipitation

1. Add 300 µl of Isopropanol to the sample from the Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 x g for 5 minutes.
3. Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 x g for 3 minutes.
5. Discard the supernatant and air-dry the pellet for 10 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the Buffer E and incubate at 60°C for 5-10 minutes to dissolve the DNA pellet. During the incubation, tap the bottom of the tube to promote DNA rehydration.

Column System (DNA Pure) Protocol

＊Add 60ml of the absolute ethanol to the Buffer W2 prior to initial use.
＊Pre-heat the Buffer E to 60°C prior to use.

Step 1 Sample Preparation

1. Add 400 µl of the Buffer BD to the sample from Step 3 Protein Removal and shake vigorously.

Step 2 DNA Binding

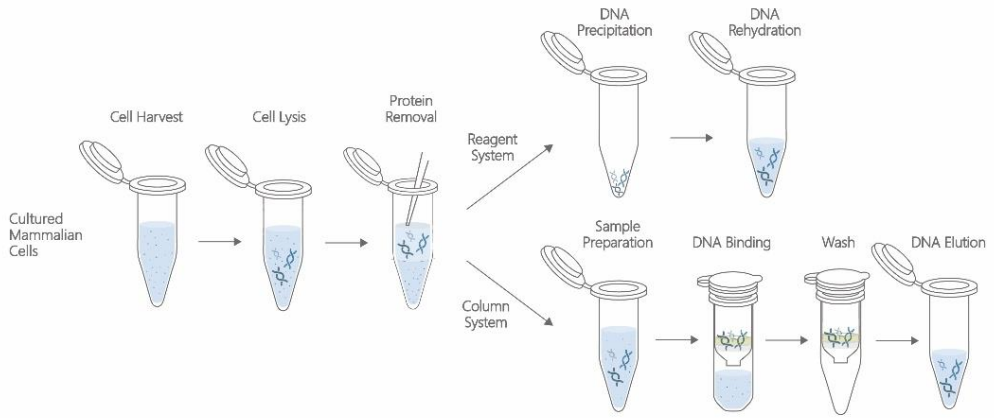
1. Place a Column DG in a 2 ml Collection Tube.
2. Transfer the sample mixture from the previous step to the Column DG.
3. Centrifuge at 14-16,000 x g for 30 seconds.
4. Discard the flow-through and place the Column DG back in the same Collection Tube.

Step 3 Wash

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

Step 4 DNA Elution

1. Place the dried Column DG in a clean 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer E or TE (not provided) into the center of the column matrix.
3. Let it stand at 60°C for 5 minutes.
4. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Dual Genomic DNA Isolation Kit Protocol

Gram-Negative Bacterial Cells Reagent System Protocol

Step 1 Sample Cells Harvesting

1. Transfer cultured bacterial cells (up to 10^9) 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 the Buffer RL by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of the Buffer CL 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. During the incubation, invert the tube every 3 minutes.

Optional Step:

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

3. 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 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 x g for 3 minutes.
4. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

Switch Step

◆If more pure DNA is required, please switch to **Column System (DNA Pure) Protocol.**

Step 4 DNA Precipitation

1. Add 300 µl of Isopropanol to the sample from the Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 x g for 5 minutes.
3. Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 x g for 3 minutes.
5. Discard the supernatant and air-dry the pellet for 10 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the Buffer E and incubate at 60°C for 5-10 minutes to dissolve the DNA pellet. During the incubation, tap the bottom of the tube to promote DNA rehydration.

Column System (DNA Pure) Protocol

＊Add 60ml of the absolute ethanol to the Buffer W2 prior to initial use.
＊Pre-heat the Buffer E to 60°C prior to use.

Step 1 Sample Preparation

1. Add 400 µl of the Buffer BD to the sample from Step 3 Protein Removal and shake vigorously.

Step 2 DNA Binding

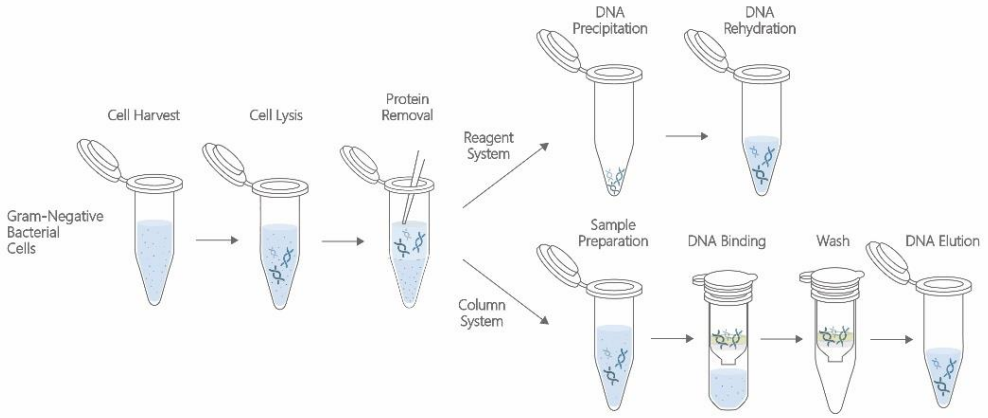
1. Place a Column DG in a 2 ml Collection Tube.
2. Transfer the sample mixture from the previous step to the Column DG.
3. Centrifuge at 14-16,000 x g for 30 seconds.
4. Discard the flow-through and place the Column DG back in the same Collection Tube.

Step 3 Wash

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

Step 4 DNA Elution

1. Place the dried Column DG in a clean 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer E or TE (not provided) into the center of the column matrix.
3. Let it stand at 60°C for 5 minutes.
4. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Dual Genomic DNA Isolation Kit Protocol

Gram-Positive Bacterial Cells Reagent System Protocol

Step 1 Sample Cells Harvesting

1. Transfer cultured bacterial cells (up to 10^9) 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.
4. Incubate at room temperature for 20 minutes.

Step 2 Lysis

1. Add 300 µl of the Buffer CL 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. During the incubation, invert the tube every 3 minutes.

Optional Step:

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

3. 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 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 x g for 3 minutes.
4. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

Switch Step

◆If more pure DNA is required, please switch to **Column System (DNA Pure) Protocol.**

Step 4 DNA Precipitation

1. Add 300 µl of Isopropanol to the sample from the Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 x g for 5 minutes.
3. Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 x g for 3 minutes.
5. Discard the supernatant and air-dry the pellet for 10 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the Buffer E and incubate at 60°C for 5-10 minutes to dissolve the DNA pellet. During the incubation, tap the bottom of the tube to promote DNA rehydration.

Column System (DNA Pure) Protocol

＊Add 60ml of the absolute ethanol to the Buffer W2 prior to initial use.
＊Pre-heat the Buffer E to 60°C prior to use.

Step 1 Sample Preparation

1. Add 400 µl of the Buffer BD to the sample from Step 3 Protein Removal and shake vigorously.

Step 2 DNA Binding

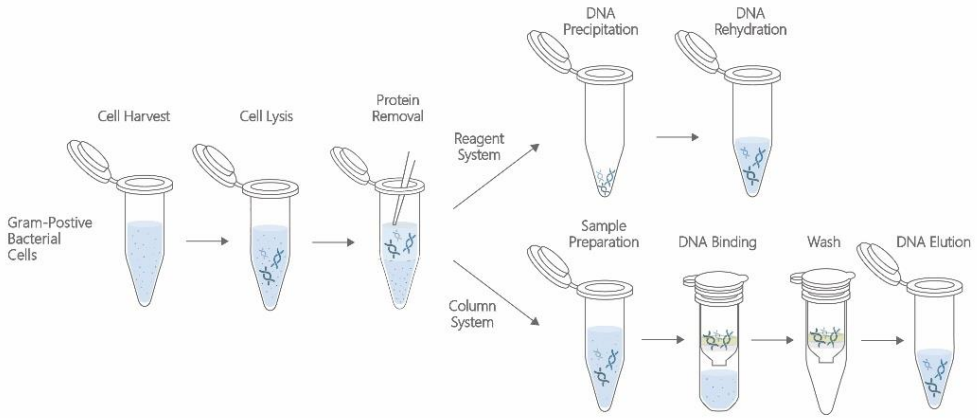
1. Place a Column DG in a 2 ml Collection Tube.
2. Transfer the sample mixture from the previous step to the Column DG.
3. Centrifuge at 14-16,000 x g for 30 seconds.
4. Discard the flow-through and place the Column DG back in the same Collection Tube.

Step 3 Wash

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

Step 4 DNA Elution

1. Place the dried Column DG in a clean 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer E or TE (not provided) into the center of the column matrix.
3. Let it stand at 60°C for 5 minutes.
4. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Dual Genomic DNA Isolation Kit Protocol

Fungus Cells Reagent System Protocol

Step 1 Sample Cells Harvesting

1. Transfer fungus cells (up to 10^8) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 x g for 5 minutes.
3. Remove the supernatant completely and resuspend the cells in 600 µl of sorbitol Buffer by pipetting the pellet.
4. Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 minutes.
5. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast.
6. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer RL by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of the Buffer CL 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. During the incubation, invert the tube every 3 minutes.

Optional Step:

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

3. 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 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 x g for 3 minutes.
4. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

Switch Step

◆If more pure DNA is required, please switch to **Column System (DNA Pure) Protocol.**

Step 4 DNA Precipitation

1. Add 300 µl of Isopropanol to the sample from the Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 x g for 5 minutes.
3. Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 x g for 3 minutes.
5. Discard the supernatant and air-dry the pellet for 10 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the Buffer E and incubate at 60°C for 5-10 minutes to dissolve the DNA pellet. During the incubation, tap the bottom of the tube to promote DNA rehydration.

Column System (DNA Pure) Protocol

＊Add 60ml of the absolute ethanol to the Buffer W2 prior to initial use.
＊Pre-heat the Buffer E to 60°C prior to use.

Step 1 Sample Preparation

1. Add 400 µl of the Buffer BD to the sample from Step 3 Protein Removal and shake vigorously.

Step 2 DNA Binding

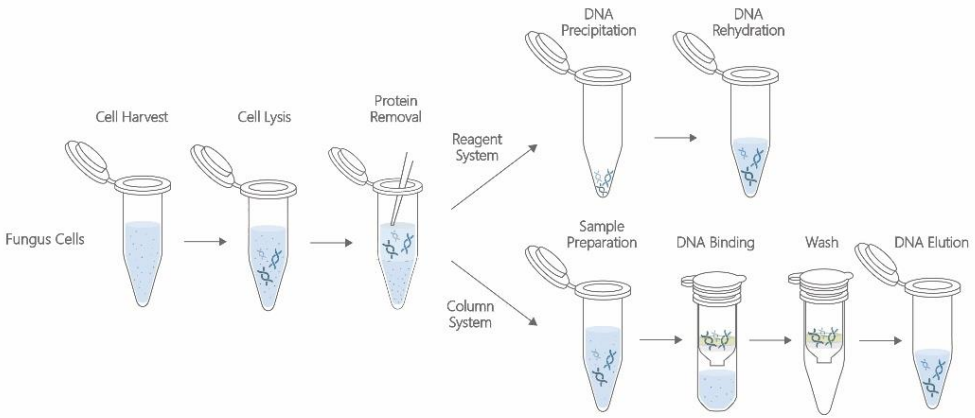
1. Place a Column DG in a 2 ml Collection Tube.
2. Transfer the sample mixture from the previous step to the Column DG.
3. Centrifuge at 14-16,000 x g for 30 seconds.
4. Discard the flow-through and place the Column DG back in the same Collection Tube.

Step 3 Wash

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

Step 4 DNA Elution

1. Place the dried Column DG in a clean 1.5 ml microcentrifuge tube (DNase & RNase free).
2. Add 50-200 µl of Pre-Heated Buffer E or TE (not provided) into the center of the column matrix.
3. Let it stand at 60°C for 5 minutes.
4. Centrifuge for 2 minutes at 14-16,000 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	Decrease the sample amounts prior to use.
	Ethanol not added	Add the absolute ethanol (see the bottle label for volume) to the Buffer W2 prior to the initial use.
	Ethanol not added to the lysate	Make sure that the ethanol was added to the lysate before applying the sample to the Column DG.
	Buffer E pH is too low	Check the pH.
	Buffer E not pre-heated at 60°C	Pre-heat the Elution Buffer to 60°C prior to use.
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	Store mammalian tissues at -80°C and bacteria at -20°C until use. The whole blood can be stored at 4°C for no longer than 1~2 days.
Inhibition of downstream enzymatic reactions	Purified DNA containing residual	If the residual solution is seen in the purification column after washing the column with the Buffer W2, empty the collection tube and re-spin the column for an additional 1 min. at the maximum speed (≥12000 x g).
	Purified DNA contains residual	Use the correct order for the Wash Buffers. Always wash the purification column with the Buffer W1 first, and then proceed to the wash with the Buffer W2

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- Buffers W1 contain irritants. Wear gloves when handling these buffers.
- Add 60 ml of the ethanol to the Buffer W2 before use.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.