DUAL Genomic DNA Isolation Kit
(Blood/Cultured Cell/Fungus)

Cat. No.: SN015-0100 Size: 100 Reactions
Cat. No.: SN015-0004 Size: 4 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 10^8)
Format: Reagent and spin column
Yield: Up to 50 µg
Operation time: Within 60 minutes
Elution volume: 50～200 µl

Description

The DUAL Genomic DNA Isolation Kit (Blood/ Cultured Cell/ Fungus) is designed to combine the reagent system and spin column system. The kit could be used to isolate the genomic DNA from the whole blood, frozen blood, buffy coat, cultured animal/ bacterial cells, and fungus. This unique reagent system ensures the 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 use in PCR or other enzymatic reactions.

Features

- Fast, reproducible and easy processing by using reagent or spin column system.
- To isolate high quality genomic DNA.
- Isolated genomic DNA is compatible with various downstream applications.

Applications

- Restriction enzyme digestion.
- PCR amplification.
- Southern blotting.
- Real-Time PCR assay .

Kit Contents

Contents	SN015-0100	SN015-0004
Buffer RL	100 ml X 1 bottle	2 ml X 2 vials
Buffer CL	35 ml X 1 bottle	1.5 ml X 1 vial
Buffer PO	12 ml X 1 bottle	0.5 ml X 1 vial
Buffer BD	45 ml X 1 bottle	2 ml X 1 vial
Buffer W1	45 ml X 1 bottle	2 ml X 1 vial
Buffer W2 (Add ethanol)	15 ml X 1 bottle (60 ml X 1 bottle)	0.3 ml X 2 vials (1.2 ml X 2 vials)
Buffer BE	10 ml X 1 bottle	1 ml X 1 vial
DG Column	50 pieces X 2 bags	4 pieces X 1 bag
Collection Tube	50 pieces X 2 bags	

Quality Control

The quality of the DUAL 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
- RNase A (10 mg/ ml)
- Lyticase or zymolase (for fungus)
- Water bath / Dry bath
- Isopropanol
- β- mercaptoethanol
- Absolute ethanol

Buffer Preparation

- TFor Gram-positive Bacteria: Lysozyme Buffer: 20 mg/ml lysozyme, 20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100, pH8.0.
- For fungus: Sorbitol buffer: 1.2 M sorbitol, 10mM $CaCl_2$, 0.1M Tris-HCl, pH7.5, 35 mM β-mercaptoethanol.
- TE buffer, pH8.0 (Selective): 10 mM Tris-HCl, pH 8.0 with 1 mM EDTA

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 the blood (up to 300 µl) or buffy coat(up to 200 µl) 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

7. Add 300 µl of the Buffer CL to the resuspended cells from Step 1 and mix by vortex.
8. 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:

9. 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

10. Add 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
11. Incubate on ice for 5 minutes.
12. Centrifuge at 14-16,000 x g for 3 minutes.
13. 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

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

Step 5 DNA Rehydration

19. Add 50-100 µl of the Buffer BE 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

- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- Pre-heat the Buffer BE to 60°C prior to use.

Step 4 Sample Preparation

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

Step 5 DNA Binding

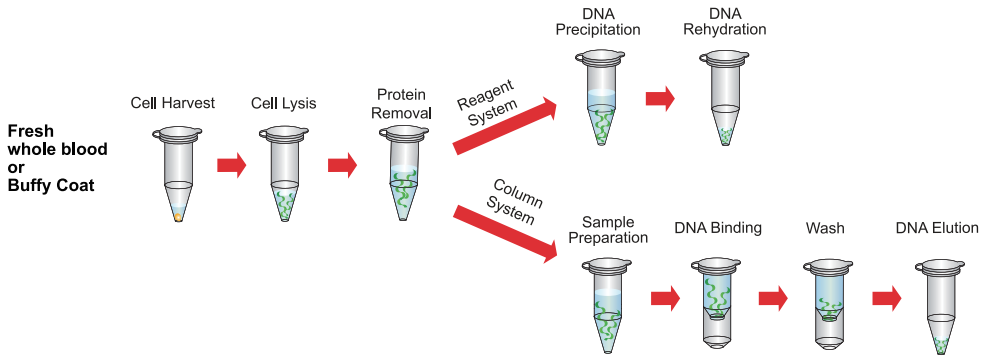
15. Place a DG Column in a 2 ml Collection Tube.
16. Transfer the sample mixture from the previous step to the DG Column.
17. Centrifuge at 14-16,000 x g for 30 seconds.
18. Discard the flow-through and place the DG Column back in the 2 ml Collection Tube.

Step 6 Wash

19. Add 400 µl of the Buffer W1 into the DG Column.
20. Centrifuge at 14,000 x g for 30 seconds.
21. Discard the flow-through and place the DG Column back into the same Collection tube.
22. Add 600 µl of the Buffer W2 (Ethanol added) into the DG Column.
23. Centrifuge at 14,000 x g for 30 seconds.
24. Discard the flow-through and place the DG Column back into the same Collection tube.
25. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 7 DNA Elution

26. Transfer the dried DG Column to a new 1.5 ml microcentrifuge tube.
27. Add 50-200 µl of Pre-Heated Buffer BE or TE (not provided) into the center of the column matrix.
28. Let it stand at 60°C for 5 minutes.
29. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



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

4. Add 300 µl of the Buffer CL to the resuspended cells from Step 1 and mix by vortex.
5. 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:

6. 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

7. Add 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
8. Incubate on ice for 5 minutes.
9. Centrifuge at 14-16,000 x g for 3 minutes.
10. 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

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

Step 5 DNA Rehydration

16. Add 50-100 µl of the Buffer BE 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

- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- Pre-heat the Buffer BE to 60°C prior to use.

Step 4 Sample Preparation

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

Step 5 DNA Binding

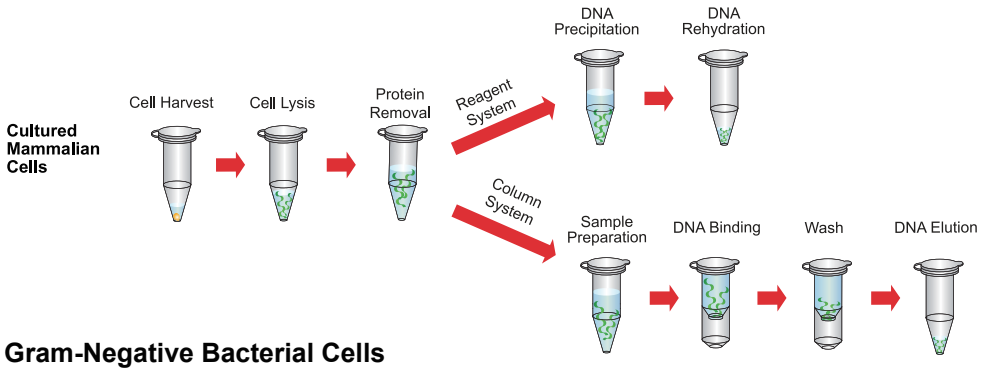
12. Place a DG Column in a 2 ml Collection Tube.
13. Transfer the sample mixture from the previous step to the DG Column.
14. Centrifuge at 14-16,000 x g for 30 seconds.
15. Discard the flow-through and place the DG Column back in the 2 ml Collection Tube.

Step 6 Wash

16. Add 400 µl of the Buffer W1 into the DG Column.
17. Centrifuge at 14,000 x g for 30 seconds.
18. Discard the flow-through and place the DG Column back into the same Collection tube.
19. Add 600 µl of the Buffer W2 (Ethanol added) into the DG Column.
20. Centrifuge at 14,000 x g for 30 seconds.
21. Discard the flow-through and place the DG Column back into the same Collection tube.
22. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 7 DNA Elution

23. Transfer the dried DG Column to a new 1.5 ml microcentrifuge tube.
24. Add 50-200 µl of the Pre-Heated Buffer BE or TE (not provided) into the center of the column matrix.
25. Let it stand at 60°C for 5 minutes.
26. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



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

4. Add 300 µl of the Buffer CL to the resuspended cells from Step 1 and mix by vortex.
5. 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:

6. 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

7. Add 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
8. Incubate on ice for 5 minutes.
9. Centrifuge at 14-16,000 x g for 3 minutes.
10. 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

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

Step 5 DNA Rehydration

16. Add 50-100 µl of the Buffer BE 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

- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- Pre-heat the Buffer BE to 60°C prior to use.

Step 4 Sample Preparation

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

Step 5 DNA Binding

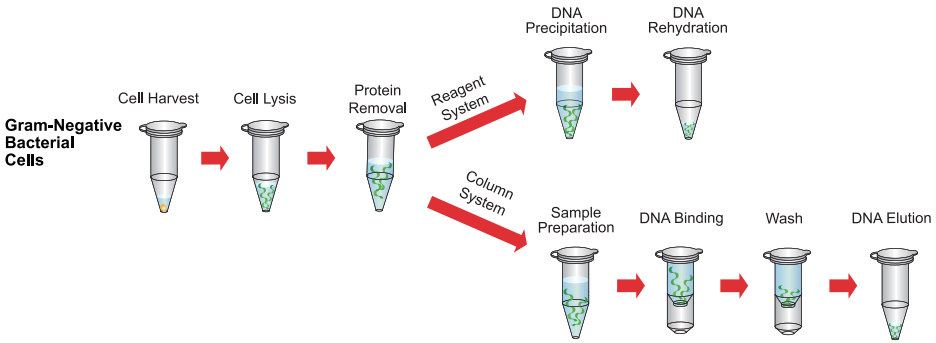
12. Place a DG Column in a 2 ml Collection Tube.
13. Transfer the sample mixture from the previous step to the DG Column.
14. Centrifuge at 14-16,000 x g for 30 seconds.
15. Discard the flow-through and place the DG Column back in the 2 ml Collection Tube.

Step 6 Wash

16. Add 400 µl of the Buffer W1 into the DG Column.
17. Centrifuge at 14,000 x g for 30 seconds.
18. Discard the flow-through and place the DG Column back into the same Collection tube.
19. Add 600 µl of the Buffer W2 (60 ml Ethanol added) into the DG Column.
20. Centrifuge at 14,000 x g for 30 seconds.
21. Discard the flow-through and place the DG Column back into the same Collection tube.
22. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 7 DNA Elution

23. Transfer the dried DG Column to a new 1.5 ml microcentrifuge tube.
24. Add 50-200 µl of Pre-Heated Buffer BE or TE (not provided) into the center of the column matrix.
25. Let it stand at 60°C for 5 minutes.
26. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Gram-Postive 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

5. Add 300 µl of the Buffer CL to the resuspended cells from Step 1 and mix by vortex.
6. 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:

7. 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

8. Add 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
9. Incubate on ice for 5 minutes.
10. Centrifuge at 14-16,000 x g for 3 minutes.
11. 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

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

Step 5 DNA Rehydration

17. Add 50-100 µl of the Buffer BE 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

◆ When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
◆ When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
◆ Pre-heat the Buffer BE to 60°C prior to use.

Step 4 Sample Preparation

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

Step 5 DNA Binding

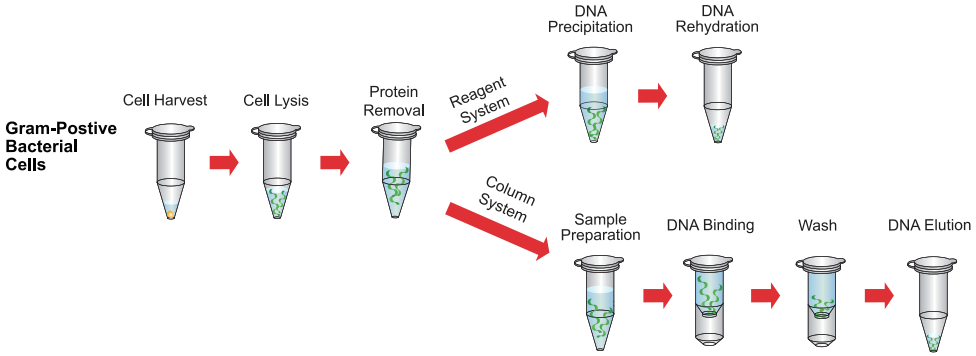
13. Place a DG Column in a 2 ml Collection Tube.
14. Transfer the sample mixture from the previous step to the DG Column.
15. Centrifuge at 14-16,000 x g for 30 seconds.
16. Discard the flow-through and place the DG Column back in the 2 ml Collection Tube.

Step 6 Wash

17. Add 400 µl of the Buffer W1 into the DG Column.
18. Centrifuge at 14,000 x g for 30 seconds.
19. Discard the flow-through and place the DG Column back into the same Collection tube.
20. Add 600 µl of the Buffer W2 (Ethanol added) into the DG Column.
21. Centrifuge at 14,000 x g for 30 seconds.
22. Discard the flow-through and place the DG Column back into the same Collection tube.
23. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 7 DNA Elution

24. Transfer the dried DG Column to a new 1.5 ml microcentrifuge tube.
25. Add 50-200 µl of Pre-Heated Buffer BE or TE (not provided) into the center of the column matrix.
26. Let it stand at 60°C for 5 minutes.
27. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



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

7. Add 300 µl of the Buffer CL to the resuspended cells from Step 1 and mix by vortex.
8. 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:

9. 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
10. Add 100 µl of the Buffer PO to the sample lysate and vortex immediately for 10 seconds.
11. Incubate on ice for 5 minutes.
12. Centrifuge at 14-16,000 x g for 3 minutes.
13. 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
14. Add 300 µl of Isopropanol to the sample from the Step 3 and mix well by inverting 20 times.
15. Centrifuge at 14-16,000 x g for 5 minutes.
16. Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet.
17. Centrifuge at 14-16,000 x g for 3 minutes.
18. Discard the supernatant and air-dry the pellet for 10 minutes.

Step 5 DNA Rehydration
19. Add 50-100 µl of the Buffer BE 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.

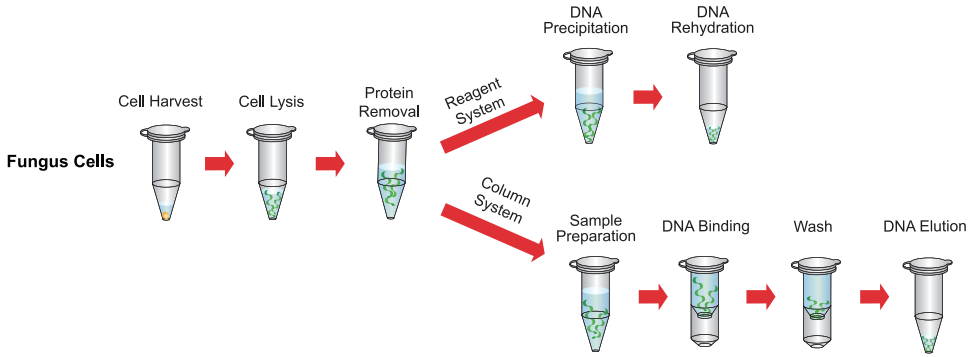
Column System (DNA Pure) Protocol
◆ When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
◆ When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
◆ Pre-heat the Buffer BE to 60°C prior to use.

Step 4 Sample Preparation
14. Add 400 µl of the Buffer BD to the sample from the Step 3 Protein Removal and shake vigorously.

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

Step 6 Wash
19. Add 400 µl of the Buffer W1 into the DG Column.
20. Centrifuge at 14,000 x g for 30 seconds.
21. Discard the flow-through and place the DG Column back into the same Collection tube.
22. Add 600 µl of the Buffer W2 (60 ml Ethanol added) into the DG Column.
23. Centrifuge at 14,000 x g for 30 seconds.
24. Discard the flow-through and place the DG Column back into the same Collection tube.
25. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 7 DNA Elution
26. Transfer the dried DG Column to a new 1.5 ml microcentrifuge tube.
27. Add 50-200 µl of Pre-Heated Buffer BE or TE (not provided) into the center of the column matrix.
28. Let it stand at 60°C for 5 minutes.
29. 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 you did genomic DNA isolation with the kit.

Problem	Cause	Solution
Low Yield of DNA	Incomplete lysed sample	Increase the sample amounts prior to use.
	Ethanol not added	Add the absolute ethanol 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 DG Column.
	Buffer BE pH is too low	Check the pH.
	Buffer BE 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 conditions	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 ethanol	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 salt.	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.

Related Ordering Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM200-0100	PCR SUPERMIX	100 Reactions
SM201-0100	Hot Start SUPERMIX	100 Reactions
SA001-0500	AGAROSE Tablet, 0.5g	100 Tablets
SL001-1000	Novel Juice Supplied in 6X Loading Buffer	1 ml
SD003-R600	100 bp DNA Ladder H3 RTU	600 µl
SD010-R600	1 Kb DNA Ladder RTU	600 µl
SD013-R600	XLarge DNA Ladder RTU	600 µl
ST040-4000	100 mM dNTP Set	4 x 1 ml
ST046-1000	100 mM dNTP Set	4 x 250 µl
ST025-1000	2.5 mM dNTP Mix	1 ml
ST010-1000	10 mM dNTP Mix	1 ml

Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents.
- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- All products are for research use only.