Genomic DNA Isolation Kit (Blood/ Cultured Cell)

Cat. No.: SN028-0100 Size: 100 Reactions
Cat. No.: SN028-0004 Size: 4 Reactions
Sample: up to 200 µl of whole blood,
up to 200 µl of buffy coat,
up to 200 µl of plasma,
up to 200 µl of serum,
up to 200 µl of body fluids,
up to 5×10^6 lymphocytes
up to 5X 10^6 cultured cells,
up to 5×10^6 of bacterial cells
Format: spin column
Yield: Up to 50 µg
Operation time: Within 60 minutes
Elution volume: 50~200 µl

Description

The spin-column based Genomic DNA Isolation Kit (Blood/ Cultured Cell) was designed specifically for genomic DNA isolation from whole blood, frozen blood, buffy coat, cultured animal and bacterial cells.Its unique buffer system ensures genomic DNA with high yield and good quality from samples while the spin column purifies and concentrates genomic DNA products previously isolated with the buffer system. The entire procedure can be completed in 1 hour without phenol/chloroform extraction needs. Purified genomic DNA is suitable for use in PCR or other enzymatic reactions.

Features

- 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

Applications

- Gene cloning
- PCR
- SNP genotyping

Kit Contents

Contents	SN028-0100	SN028-0004
Buffer CK	25 ml	2 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	0.3 ml x 2 (1.2 ml x 2)
Buffer BE	10 ml	1 ml
Column CC	2 X 50 pcs / pk	4 pcs
Collection Tubes	2 X 50 pcs / pk	4 pcs
Proteinase K (Add ddH_2O)	40 mg (2 ml)	100 µl (added)

Quality Control

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

Required Materials

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

Genomic DNA Isolation Kit Protocol

Fresh whole blood, buffy coat plasma, serum, body fluids, lymphocytes

Step 1 Sample Cells Harvesting

1. Collect blood in the EDTA-Na_2 treated collection tubes (or other anticoagulant mixtures).
2. Transfer up to 200 µl of the blood or 200 µl of buffy coat to a sterile 1.5 ml microcentrifuge tube.

Step 2 Lysis

3. Add 20 µl of proteinase K and 200 µl of Buffer CK to the sample from Step 1 and mix by pulse-vortexing for 15 seconds.
4. Incubate at 55°C for 10 minutes. During incubation, invert the tube every 3 minutes.
   #Pre-heat the Buffer BE to 60°C for Step 5 DNA Elution.

Optional Step:

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

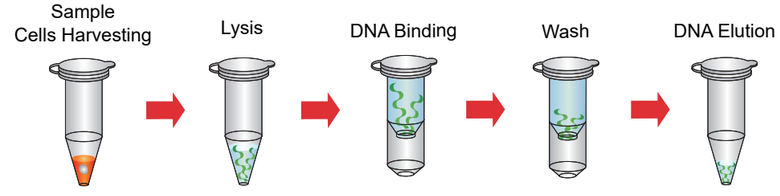
6. Add 350 µl of ethanol (96-100%) to the sample from Step 2 and mix by pulse- vortexing for 15 seconds.
7. Place a Column CC in a 2 ml Collection Tube.
8. Transfer the mixture completely to the Column CC.
9. Centrifuge at 14,000 x g for 30 seconds.
10. Discard the flow-through and place the Column CC back in the 2 ml Collection Tube.

Step 4 Wash

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

Step 5 DNA Elution

18. Transfer the dried Column CC to a new 1.5 ml microcentrifuge tube.
19. Add 50-200 µl of Pre-Heated Buffer BE or TE into the center of the column matrix.
20. Let stand at 55°C for 3 minutes.
21. Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Cultured mammalian cells

Step 1 Sample Cells Harvesting

1. Transfer the cultured mammalian cells (up to 5×10^6) to a sterile 1.5 ml microcentrifuge tube.

Step 2 Lysis

2. Add 20 µl of proteinase K and 200 µl of Buffer CK to the sample from Step 1 and mix by pulse-vortexing for 15 seconds.
3. Incubate at 55°C for 10 minutes. During incubation, invert the tube every 3 minutes.
   #Pre-heat the Buffer BE to 60°C for Step 5 DNA Elution.

Optional Step:

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

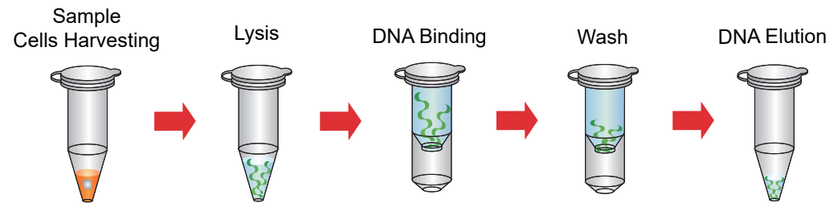
5. Add 350 µl of ethanol (96-100%) to the sample from Step 2 and mix by pulse-vortexing for 15 seconds.
6. Place a Column CC in a 2 ml Collection Tube.
7. Transfer the mixture completely to the Column CC.
8. Centrifuge at 14,000 x g for 30 seconds.
9. Discard the flow-through and place the Column CC back in the 2 ml Collection Tube.

Step 4 Wash

10. Add 400 µl of Buffer W1 into the Column CC.
11. Centrifuge at 14,000 x g for 30 seconds.
12. Discard the flow-through and place the Column CC back into the same Collection tube.
13. Add 600 µl of Buffer W2 (Ethanol added) into the Column CC.
14. Centrifuge at 14,000 x g for 30 seconds.
15. Discard the flow-through and place the Column CC back into the same Collection tube.
16. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 5 DNA Elution

17. Transfer the dried Column CC to a new 1.5 ml microcentrifuge tube.
18. Add 50-200 µl of Pre-Heated Buffer BE or TE into the center of the column matrix.
19. Let stand at 55°C for 3 minutes.
20. 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 the cultured bacterial cells (up to 5×10^6) to a sterile 1.5 ml microcentrifuge tube.

Step 2 Lysis

2. Add 20 µl of proteinase K and 200 µl of Buffer CK to the sample from Step 1 and mix by pulse-vortexing for 15 seconds.
3. Incubate at 55°C for 10 minutes. During incubation, invert the tube every 3 minutes.
   #Pre-heat the Buffer BE to 60°C for Step 5 DNA Elution.

Optional Step:

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

4. 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 DNA Binding

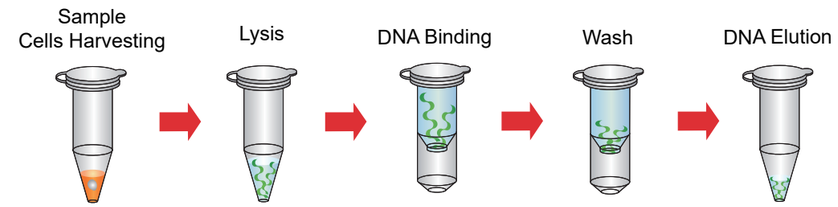
5. Add 350 µl of ethanol (96-100%) to the sample from Step 2 and mix by pulse-vortexing for 15 seconds.
6. Place a Column CC in a 2 ml Collection Tube.
7. Transfer the mixture completely to the Column CC.
8. Centrifuge at 14,000 x g for 30 seconds.
9. Discard the flow-through and place the Column CC back in the 2 ml Collection Tube.

Step 4 Wash

10. Add 400 µl of Buffer W1 into the Column CC.
11. Centrifuge at 14,000 x g for 30 seconds.
12. Discard the flow-through and place the Column CC back into the same Collection tube.
13. Add 600 µl of Buffer W2 (Ethanol added) into the Column CC.
14. Centrifuge at 14,000 x g for 30 seconds.
15. Discard the flow-through and place the Column CC back into the same Collection tube.
16. Centrifuge at 14,000 x g again for 2 minutes to remove residual Buffer W2.

Step 5 DNA Elution

17. Transfer the dried Column CC to a new 1.5 ml microcentrifuge tube.
18. Add 50-200 µl of Pre-Heated Buffer BE or TE into the center of the column matrix.
19. Let stand at 55°C for 3 minutes.
20. 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 DNase.
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.

Related Ordering Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM203-0100	OnePCR™	100 Reactions
SD110-0100	OneMARK B	600 ul
SL001-1000	Novel Juice (Supplied in 6X Loading Buffer)	1 ml

Caution

1. During operation, always wear a lab coat, disposable gloves, and protective equipment.
2. After receiving the kit, store the vial of Proteinase K at 4°C for long term storage.
3. Check buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
4. When using 100 reaction assays , add 60 ml of the ethanol (96–100%) to the Buffer W2, and shake before use.
5. When using 4 reaction assays, add 1.2 ml of the ethanol (96–100%) to the each vial of Buffer W2, and shake before use.
6. For 100 reaction assays, add DNase-Free Water to the proteinase K, vortex to dissolve and shake
7. Buffers W1 contain irritants. Wear gloves when handling these buffers.
8. All products are for research use only.