ISO 9001/14001 Certified Company

G-spin™ Genomic DNA Extraction Kit [for Bacteria]

Cat. No. 17121 50 Columns

DESCRIPTION

G-spin™ Genomic DNA Extraction Kit are designed for rapid isolation of genomic DNA from a variety of sample sources including fresh or frozen animal cells/tissues (for Cell/Tissue) and Gram-negative & Gram-positive bacteria (for Bacteria), yeast (for Yeast), or bloods (for Blood). The purified DNA is free of contaminants and impurities and is ideal for all PCR, Southern blotting, RAPD, and sequencing applications.

G-spin™ kit uses advanced silica-gel membrane technology for rapid and efficient purification of genomic DNA without organic extraction or ethanol precipitation. Furthermore, G-spin™ buffer system is optimized to allow rapid and simple cell lysis followed by selective binding of DNA to the column. G-spin™ procedure is very simple, so you can purify DNA from a variety of target source within 20-40min.

KIT CONTENTS

Label	Description	Contain
Pre-Buffer[1]	for Gram Positive Bacteria	3 ml
G-Buffer[2]	for Gram Positive and Negative Bacteria	20 ml
Binding Buffer[3]		15 ml
Washing Buffer A	add 21 ml of absolute EtOH	9 ml
Washing Buffer B	add 40 ml of absolute EtOH	10 ml
Elution Buffer		20 ml
Columns	columns containing silica-membrane	50 columns
Collection tubes	polypropylene tube for 2 ml	50 tubes
RNase A[4] (Lyophilized powder)	dissolve in 300 µl of DW	3 mg
Proteinase K[5](Lyophilized powder)	dissolve in 88 µl of DW	1.76 mg
Lysozyme[6] (Lyophilized powder)	dissolve in 200 µl of DW	20 mg

1. **Pre-Buffer** : Before use, **add 30 µl of RNase A Solution**. After add RNase A, Pre-Buffer should then **be stored at 2-8°C**.
2. **G-Buffer** : Before use, **add 250 µl of RNase A Solution and add 40 µl of Proteinase K solution**. After add RNase A and Proteinase K, G-Buffer should then **be stored at 2-8°C**.
3. **Binding Buffer** : Genomic DNA binding solution. After receiving, Binding Buffer should then **be store at 4°C.**
4. **Lyophilized RNase A** : **Dissolve the RNase A in 300 µl of pure D.W. to each vial**. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized RNase A can only be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.
5. **Lyophilized Proteinase K** : **Dissolve the Proteinase K in 88 µl of pure D.W. to each vial**. The lyophilized Proteinase K can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized Proteinase K can only be dissolved in D.W.; dissolved Proteinase K should be immediately stored at -20℃. The Proteinase K solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.
6. **Lyophilized Lysozyme** : **Dissolve the Lysozyme in 200 µl of pure D.W. to each vial**. The lyophilized Lysozyme can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized Lysozyme can only be dissolved in D.W.; dissolved Lysozyme should be immediately stored at -20℃. The Lysozyme solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

STORAGE

- Pre-Buffer : store at 4°C.
- G-Buffer : store at 4°C. G-Buffer has effective efficiency for two months at RT.
- Binding Buffer : store at 4°C.
- Washing Buffer A, B : store at RT .
- RNase A Soln, Proteinase K Soln, Lysozyme Soln. : store at -20°C .
- The other components : store at RT

HARVEST CELLS

1. *For bacteria* : Harvest cells in a microcentrifuge tube by centrifuging for 1min at 13,000rpm. Collected cell are resuspended by vortex or repetitive tapping.

PROTOCOL (For Gram-Negative Bacteria)

1. Harvest 1-2 ml of cells (OD_{600} : 0.8-1.0) by centrifuging at 13,000rpm for 1min. Remove supernatant.
   Note : After centrifugation, remove the supernatant and completely resuspend by vortex or tapping. Do not to overload the sample.
2. Add 300 µl of G-Buffer solution, and invert-mix well.
3. Incubate at 65°C for 15 min.
   Note : To help lysis cells, invert mix the tube every 5 min during the incubation.
4. Add 250 µl of Binding Buffer, and completely mix well by pipetting (at least 10 times) or gently vortexing.
   Note : This step conduces to pass efficiently cell lysates through a column and increase gDNA binding onto column resins and important for efficient deproteinization.
5. Cell lysates loading on column and centrifuge at 13,000 rpm for 1 min.
   Note : The maximum volume of the column reservoirs 800 µl. For sample volumes of more than 800 µl, sample load and spin again.
6. To wash, add 500 µl of Washing Buffer A to column and centrifuge for 1 min at 13,000 rpm.
7. Remove solution. Add 500 µl of Washing Buffer B to column and centrifuge for 1 min at 13,000 rpm.
8. Remove solution and centrifuge for 1min at 13,000 rpm.
9. Place the G-spin™ column in a clean 1.5ml microcentrifuge tube (not provided), and add 50-200 µl of Elution Buffer directly onto the membrane.
10. Incubate at RT for 1 min, and then centrifuge for 1 min at 13,000 rpm.

PROTOCOL (For Gram-Positive Bacteria)

1. Harvest 1-2 ml of cells (OD_{600} : 0.8-1.0) by centrifuging at 13,000 rpm for 1 min. Remove supernatant.
   Note : After centrifugation, remove the supernatant and completely resuspend by vortex or tapping. Do not to overload the sample.
2. Add 50 µl of Pre-Buffer and 3 µl of Lysozyme solution, mix well.
3. Incubate at 37°C for at least 15 min.
   Note : To help lyse cells, invert mix the tube every 5 min during the incubation.
4. Add 250 µl of G-Buffer solution, and invert-mix well.
5. Incubate at 65°C for 15min.
   Note : To help lysis cells, invert mix the tube every 5min during the incubation.
6. Add 250 µl of Binding Buffer, and completely mix well by pipetting (at least 10 times) or gently vortexing.
   Note : This step conduces to pass efficiently cell lysates through a column and increase gDNA binding onto column resins and important for efficient deproteinization.
7. Cell lysates loading on column and centrifuge at 13,000 rpm for 1 min.
   Note : The maximum volume of the column reservoirs 800 µl. For sample volumes of more then 800 µl, sample load and spin again.
8. To wash, add 500 µl of Washing Buffer A to column and centrifuge for 1 min at 13,000 rpm.
9. Remove solution. Add 500 µl of Washing Buffer B to column and centrifuge for 1 min at 13,000 rpm.
10. Remove solution and centrifuge for 1 min at 13,000 rpm.
11. Place the G-spin™ column in a clean 1.5 ml microcentrifuge tube (not provided), and add 50-200 µl of Elution Buffer directly onto the membrane.
12. Incubate at RT for 1 min, and then centrifuge for 1 min at 13,000 rpm.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• Total genomic DNA preparation from gram-negative and positive bacteria cells

G-spin™ Genomic DNA Extraction Kit for Bacteria is provides a simple and rapid method for the isolation of total DNA from gram-negative bacteria and positive bacteria.

Species and Material	Amount (OD_{600}=0.8-1.0)	Total DNA Yield (μg)	DNA Purity
E. coli	2 ml	15-20 μg	2.01
Corynebacterium spp.	2 ml	10-18 μg	2.02
Salmonella pullorum	2 ml	15-22 μg	2.01
Staphylococcus aureus	2 ml	11-19 μg	2.0

• Genomic DNA isolation from various samples

G-spin™ Genomic DNA Extraction Kit for Bacteria is ideal for purifying DNA from small amounts of starting materials. For the purpose to obtain optimum DNA yield and quality, do not to overload the sample. It is important. The volume of 1-2 ml is suitable for gDNA prep. (Ideal OD_{600} value is 0.8-1.1)

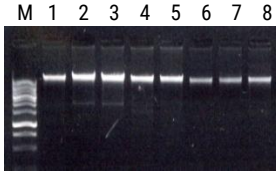

Fig. 1. Analysis of gDNA isolated from various samples with G-spin™ Genomic DNA Extraction Kit for Bacteria. 2 µl out of 200 µl of eluent was loaded.
Lane M, 1Kb Ladder DNA Marker, **lane 1,** *E. coli*, **lane 2,** *Salmonella pullorum*, **lane 3,** *Pseudomonas*, **lane 4,** *Corynebacterium spp*, **lane 5,** *Staphylococcus. aureus*, **lane 6,** *Bacillus subtilis*, **lane 7,** *Micrococcus*, **lane 8,** *Lactobacillus breris* (OD_{600} value=1.00)

• Restriction enzyme digestion for isolated genomic DNA

Ensure that the carrier DNA dose not interfere with your downstream application. Because of isolated genomic DNA was purified without contamination of protein, RNA, and so on.

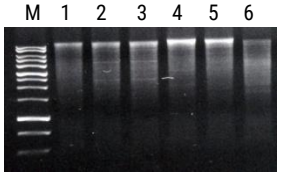

Fig. 2. Genomic DNA isolated with G-spin™ Genomic DNA prep Kit is suitable for restriction enzyme digestions.
Lane M, 1Kb Ladder DNA Marker, **lane 1**, *E. coli*, **lane 2**, *Salmonella pullorum*, **lane 3**, *Pseudomonas*, **lane 4**, *Corynebacterium spp*, **lane 5**, *Staphylococcus. aureus*, **lane 6**, *Bacillus subtilis*. Restriction enzyme: *Eco*R I, Incubation time: 2hr.

• PCR amplification of genomic DNA isolated from bacteria.

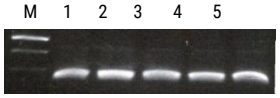

Fig. 3. Agarose gel electrophoresis of PCR products from isolated genomic DNA.
Lane M, 100bp Ladder DNA Marker; **lane 1,** *E. coli*, **lane 2,** *Salmonella pullorum*; **lane 3,** *Corynebacterium sp;* **lane 4,** *Staphylococcus. Aureus*; **lane 5,** *Bacillus subtilis*.

• Comparison of genomic DNA yield from different company kit

In order to compare of genomic DNA yield from competitive company kit, make on experiment. Yield of extraction, G-spin™ Kit stand in different levels compare of competitive company kit.

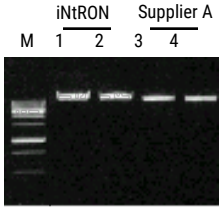

Fig. 4. Agarose gel electrophoresis of isolated genomic DNA from different company kit.
Lane 1, 2, iNtRON; **lane 3, 4,** Supplier A; **Lane M,** 1Kb Ladder DNA Marker

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low DNA yield	Too much starting material	- Check the step 1 of protocol; Reduce the amount of starting material used.
	Reagents correctly were stored	- Check the "STOAGE"; Pre-Buffer, G-Buffer and Binding Buffer shall be stored at 4°C after receiving.
	Incomplete lysis	- Lysis time and buffer volume not correct for sample size - Gram-positive bacteria : Increase pre-incub--ation time
	DNA inefficiently eluted	- Elute product with 100-200µl of the Elution Buffer to obtain best result. - Depending on starting material size, decrease or increase volume of Elution Buffer - Add Elution Buffer to the center of the G-spin™ column to ensure that the Elution Buffer completely covers the membrane.
	Washing Buffer A, B did not contain 100% EtOH	- 100% EtOH must be added to the Washing Buffer A, B before use.
DNA does not perform well in the downstream application	Ethanol carryover	- Ensure that during the Washing Buffer B, the G-spin™ column is spun at maximum speed 1min to dry the G-spin™ column