PRODUCT INFORMATION

SciPhi™ Genomic DNA Purification Kit

#NXG991-M

#NXG991-S

#NXG991-XS

Read Storage and Reagent preparation information before first use.

For Research Use Only. Not recommended for diagnostic use.

NextGen Life Sciences

Kit Components:

Component	Quantity		
	NXG991-M	NXG991-S	NXG991-XS
DNA Purification Columns with Collection Tubes	50	10	5
Collection Tubes	50	10	5
Lysis Buffer	24 ml	5 ml	1.2ml x 2
Digestion Solution	11 ml	2.5 ml	1.1 ml
Wash Buffer I (Conc.)	10 ml	2 ml	1 ml
Wash Buffer II (Conc.)	10 ml	2 ml	1 ml
Elution Buffer	30 ml	6 ml	1.5ml x 2
Ethanol	75 ml	15 ml	7.5 ml
Proteinase K	1.2 ml	240 µl	120 µl
RNase A	1 ml	200 µl	100 µl

Storage Instructions:

As long as the RNase A and proteinase K solutions are not opened, they are stable at room temperature. Once they've been opened, they need to be kept at -20 °C. Other components of the kit can be kept at room temperature (15 to 25°C).
Please remember to properly close the bag containing SciPhi™ Genomic DNA Purification Columns after each usage.

Product Description:

SciPhi™ Genomic DNA Purification Kit is suitable for fast and efficient genomic DNA purification from whole blood, mammalian cell culture, tissue samples, bacteria and yeast. This kit is based on spin column silica membrane technology. Hence, no resins, no phenol-chloroform extractions, and no alcohol precipitations are needed. It can purify DNA in less than 20 minutes after lysis with a yield of >30 µg (Table 1). Purified genomic DNA from the kit is ideal for PCR, qPCR, restriction digestion, and Southern blotting applications.

Table 1: DNA yields from various sample sources

Source	Quantity	Yield, µg
Blood	200 µl	4-6
Mouse Heart	10 mg	10-15
Mouse Tail	0.5 cm	8-10
Rat Liver	10 mg	10-20
Rat Spleen	5 mg	20-30
Rat Kidney	10 mg	25-30
Rabbit ear	20 mg	5-10
Bacillus pumilis cells	2 X 10^9 cells	10-15
Escherichia coli cells	2 X 10^9 cells	10-15
HeLa cells	2 X 10^6 cells	15-20
Jurkat cells	2 X 10^6 cells	25-30
Saccharomyces cerevisiae cells	1 X 10^8 cells	3-5

Principle:

Digestion/Lysis: Depending on the initial material, the samples are subjected to Proteinase K digestion using either the Digestion or Lysis Solution provided. This process helps break down the cells.

RNA Removal: To eliminate RNA present in the samples, they are treated with RNase A. This enzymatic treatment specifically targets and degrades RNA molecules.

Binding and Purification: The lysate (resulting mixture) is then mixed with ethanol and loaded onto a purification column. In this step, the DNA molecules present in the lysate attach to the silica membrane within the column, while impurities are effectively washed away using prepared wash buffers.

Elution: Genomic DNA is then can be eluted from the purification column under low ionic strength conditions using an Elution Buffer. This allows the DNA to be collected in a separate solution for further analysis or use.

Important Information

- Avoid repeated freeze/thaw cycle of samples to avoid DNA degradation.
- Always extract DNA from fresh sample or the samples that has been stored at -20°C or -70°C.
- Always check for salt precipitation in the **lysis buffer** and **digestion solution** before each use. Warm the solution to 37°C and then cool to 25°C before using to dissolve any precipitate.
- Handle **Wash Buffer I** and **Lysis buffer** after wearing the gloves.
- Prepare **Wash Buffer I** and **Wash Buffer II** according to Table 2 before first use:

Table 2: Instruction to prepare wash buffers

Component	NXG991-M		NXG991-S		NXG991-XS	
	Wash Buffer I	Wash Buffer II	Wash Buffer I	Wash Buffer II	Wash Buffer I	Wash Buffer II
Concentrated Wash Buffer	10 ml	10 ml	2 ml	2 ml	1 ml	1 ml
Ethanol (96-100%)	30 ml	30 ml	6 ml	6 ml	3 ml	3 ml
Total Volume	**40 ml**	**40 ml**	**8 ml**	**8 ml**	**4 ml**	**4 ml**

Additional Instruments and Consumables Required

- Pipettes and pipette tips
- Vortex Mixture
- Microcentrifuge
- Water Bath or Heat Block
- Gloves
- 1.5 ml Microcentrifuge tubes
- Buffers:
  - **For mammalian cell lysate:**
    - Phosphate Buffer Saline (PBS): 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4
    - Tris-EDTA (TE) Buffer: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA
  - **Lysis buffer for gram-positive bacteria:**
    - 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100.
    - Add lysozyme (20mg/ml) immediately before use.
  - **Lysis buffer for Yeast:**
    - 5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA

PROTOCOLS FOR DNA ISOLATION

DNA isolation protocol from mammalian tissue and rodent tail, cultured mammalian cells, mammalian blood, gram-negative, gram-positive bacteria, yeast and buccal swabs are described on page no. 5-12

DNA Isolation from Mammalian Tissue and Rodent Tail

1. Grind up to 20 mg of mammalian tissue (use up to 10 mg of spleen tissue), 0.6 cm (rat) or 0.5 cm (mouse) tail clip in liquid nitrogen using a mortar and pestle. Alternatively, chop the tissue into small pieces or use a homogenizer for disruption.
2. Transfer the ground tissue into a 1.5 ml microcentrifuge tube and add 180 µl of Digestion Solution. Mix thoroughly by vortexing or pipetting to achieve a uniform suspension.
3. Add 20 µl of Proteinase K Solution and mix again.
4. Incubate the sample at 56°C until complete lysis occurs, ensuring there are no particles left. Occasionally vortex the vial during incubation or use a shaking water bath, rocking platform, or thermomixer. The suggested incubation times are as follows:

Quantity	Suggested time
5 mg tissue (except spleen)	1 hour
10 mg tissue (except spleen)	2 hours
20 mg tissue (except spleen)	3 hours
5 mg spleen tissue	2 hours
10 mg spleen tissue	3 hours
Mouse tail (0.5 cm), rat tail (0.6 cm)	6 hours

Note: Incubation time may vary based on the tissue type and amount. In some cases, prolonged incubation up to 6-8 hours or overnight (for rodent tail) might be necessary for complete lysis.

5. Add 20 µl of RNase A Solution, mix by vortexing and incubate at room temperature for 10 minutes.
6. Add 200 µl of Lysis Buffer and vortex for 15 seconds to obtain a homogeneous mixture.
7. Add 400 µl of 50% ethanol, mix by pipetting or vortexing, and transfer the lysate to a SciPhi™ Genomic DNA Purification Column placed in a collection tube. Centrifuge the column at 6000 × g for 1 minute. Discard the collection tube with the flow-through solution and transfer the column to a new 2 ml collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ Genomic DNA Purification Columns tightly after each use!

8. Add 500 µl of Wash Buffer I (with ethanol added) and Centrifuge at 8000 × g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
9. Add 500 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at maximum speed (≥12000 × g) for 3 minutes.

   Optional: If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.

10. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
11. Elute genomic DNA by adding 200 µl of Elution Buffer to the centre of the SciPhi™ Genomic DNA Purification Column membrane. Incubate for 2 minutes at room

temperature and centrifuge at 8000 × g for 1 minute.

Note

- For maximum DNA yield, repeat the elution step with additional 200 µl of Elution Buffer.
- If more concentrated DNA is needed or DNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the Elution Buffer added to the column can be reduced to 50-100 µl. However, be aware that smaller volumes of Elution Buffer will yield a smaller final quantity of eluted DNA.

12. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

DNA Isolation from Cultured Mammalian Cells

1. **For Suspension Cells**: Pellet down up to 5 × 10^6 cells in a centrifuge tube by centrifugation at 250 × g for 5 min. Discard the supernatant and rinse pelleted cells once with PBS to remove residual medium and repeat the centrifugation step. Discard the supernatant.

   For Adherent Cells: Remove the growth medium from a culture plate containing up to 2×10^6 cells. Rinse cells once with PBS to remove residual medium. Discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells to a microcentrifuge tube and pellet them by centrifugation at 250 × g for 5 minutes. Discard supernatant.
2. Resuspend the collected cells in 200 µl of TE buffer or PBS. Add 200 µl of Lysis Buffer followed by 20 µl of Proteinase K Solution to the cell pellet. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3. Incubate the suspension at 56°C for 10 minutes or until the cells are completely lysed while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer.
4. Add 20 µl of RNase A Solution, mix by vortexing and incubate the suspension at room temperature for 10 minutes.
5. Add 400 µl of 50% ethanol and mix by pipetting or vortexing.
6. Add 400 µl of 50% ethanol, mix by pipetting or vortexing, and transfer the lysate to a SciPhi™ Genomic DNA Purification Column placed in a collection tube. Centrifuge the column at 6000 × g for 1 minute. Discard the collection tube with the flow-through solution and transfer the column to a new 2 ml collection tube.

   Note. Remember to tightly seal the bag of SciPhi™ Genomic DNA Purification Columns tightly after each use!
7. Add 500 µl of Wash Buffer I (with ethanol added) and Centrifuge at 8000 × g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
8. Add 500 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at maximum speed (≥12000 × g) for 3 minutes.

   Optional: If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
9. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
10. Elute genomic DNA by adding 200 µl of Elution Buffer to the centre of the SciPhi™ Genomic DNA Purification Column membrane. Incubate for 2 minutes at room temperature and centrifuge at 8000 × g for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with additional 200 µl of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., ≤2×10^6 cells) the volume of the Elution Buffer added to the column can be reduced to 50-100 µl. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

DNA Isolation from Mammalian Blood

1. Add 400 µl of Lysis Buffer followed by 20 µl of Proteinase K Solution to 200 µl of whole blood. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
2. Incubate the suspension at 56°C for 10 minutes or until the cells are completely lysed while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer.
3. Add 200 µl of ethanol (96-100%) and mix by pipetting or vortexing.
4. Transfer the prepared lysate to a SciPhi™ Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column at 6000 × g for 1 minute. Discard the collection tube containing the flow-through solution and transfer the column to a new 2 ml collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ Genomic DNA Purification Columns tightly after each use!
5. Add 500 µl of Wash Buffer I (with ethanol added) and centrifuge at 8000 × g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
6. Add 500 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at maximum speed (≥12000 × g) for 3 minutes.

   Optional: If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
7. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
8. Elute genomic DNA by adding 200 µl of Elution Buffer to the centre of the SciPhi™ Genomic DNA Purification Column membrane. Incubate for 2 minutes at room temperature and centrifuge at 8000 × g for 1 minute.

   Note:

   - For maximum DNA yield, repeat the elution step with additional 200 µl of Elution Buffer.
   - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., 50 µl) the volume of the Elution Buffer added to the column can be reduced to 50-100 µl. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
9. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

DNA Isolation from Gram-Negative Bacteria

1. Pellet down up to 2×10^9 bacterial cells in a 1.5 / 2 mL microcentrifuge tube by centrifugation at 5000 × g for 10 minutes and discard the supernatant
2. Resuspend the pellet in 180 µl of Digestion Solution followed by addition of 20 µl Proteinase K. Mix thoroughly by pipetting or vortexing to obtain a uniform suspension.
3. Incubate the suspension at 56°C for 30 minutes or until the cells are completely lysed while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer.
4. Add 20 µl of RNase A solution and incubate the suspension for 10 minutes at room temperature.
5. Add 200 µl of Lysis buffer and mix the suspension thoroughly by vortexing or pipetting for 15 seconds or until homogenous suspension is obtained.
6. Add 400 µl of 50% ethanol and mix by pipetting or vortexing.
7. Transfer the prepared lysate to a SciPhi™ Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column at 6000 × g for 1 minute. Discard the collection tube containing the flow-through solution and transfer the column to a new 2 ml collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ Genomic DNA Purification Columns tightly after each use!
8. Add 500 µl of Wash Buffer I (with ethanol added) and centrifuge at 8000 × g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
9. Add 500 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at maximum speed (≥12000 × g) for 3 minutes.

   Optional: If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
10. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
11. Elute genomic DNA by adding 200 µl of Elution Buffer to the centre of the SciPhi™ Genomic DNA Purification Column membrane. Incubate for 2 minutes at room temperature and centrifuge at 8000 × g for 1 minute.

    Note:

    - For maximum DNA yield, repeat the elution step with additional 200 µl of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material, the volume of the Elution Buffer added to the column can be reduced to 50-100 µl. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
12. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

DNA Isolation from Gram-Positive Bacteria

Prepare Gram-Positive bacteria lysis buffer before starting: 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100, add lysozyme to 20 mg/mL immediately before use.

1. Pellet down up to 2×10^9 bacterial cells in a 1.5 / 2 mL microcentrifuge tube by centrifugation at 5000 × g for 10 minutes and discard the supernatant
2. Resuspend the pellet in 180 µl of Gram-Positive bacteria lysis buffer followed by incubation at 37°C for 30 minutes.
3. Add 200 µl of Lysis buffer and 20 µl of Proteinase K. Mix the suspension thoroughly by vortexing or pipetting until homogenous suspension is obtained.
4. Incubate the suspension at 56°C for 30 minutes or until the cells are completely lysed while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer.
5. Add 20 µl of RNase A solution and incubate the suspension for 10 minutes at room temperature.
6. Add 400 µl of 50% ethanol and mix by pipetting or vortexing.
7. Transfer the prepared lysate to a SciPhi™ Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column at 6000 × g for 1 minute. Discard the collection tube containing the flow-through solution and transfer the column to a new 2 ml collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ Genomic DNA Purification Columns tightly after each use!
8. Add 500 µl of Wash Buffer I (with ethanol added) and centrifuge at 8000 × g for 1 minute. Discard the flow-through and place the purification column back into the collection tube
9. Add 500 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at maximum speed (≥12000 × g) for 3 minutes.

   Optional: If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
10. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
11. Elute genomic DNA by adding 200 µl of Elution Buffer to the centre of the SciPhi™ Genomic DNA Purification Column membrane. Incubate for 2 minutes at room temperature and centrifuge at 8000 × g for 1 minute.

    Note:

    - For maximum DNA yield, repeat the elution step with additional 200 µl of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material, the volume of the Elution Buffer added to the column can be reduced to 50-100 µl. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
12. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

DNA Isolation from Yeast

Prepare Yeast lysis buffer before starting: 5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA.

1. Pellet down up to 1×10^8 yeast cells in a 1.5 / 2 mL microcentrifuge tube by centrifugation at ≥12000 × g for 5-10 seconds and discard the supernatant
2. Resuspend the pellet in 500 µl of Yeast lysis buffer followed by incubation at 37°C for 1 hour.
3. Centrifuge the cell suspension at 3000 × g for 10 minutes.
4. Resuspend the pellet in 180 µl of Digestion Solution followed by addition of 20 µl Proteinase K. Mix thoroughly by pipetting or vortexing to obtain a uniform suspension.
5. Incubate the suspension at 56°C for ~45 minutes or until the cells are completely lysed while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer.
6. Add 20 µl of RNase A solution and incubate the suspension for 10 minutes at room temperature.
7. Add 200 µl of Lysis buffer and mix the suspension for thoroughly by vortexing or pipetting for 15 seconds or until homogenous suspension is obtained.
8. Add 400 µl of 50% ethanol and mix by pipetting or vortexing.
9. Transfer the prepared lysate to a SciPhi™ Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column at 6000 × g for 1 minute. Discard the collection tube containing the flow-through solution and transfer the column to a new 2 ml collection tube. **Note: Remember to tightly seal the bag of SciPhi™ Genomic DNA Purification Columns tightly after each use!**
10. Add 500 µl of Wash Buffer I (with ethanol added) and centrifuge at 8000 × g for 1 minute. Discard the flow-through and place the purification column back into the collection tube
11. Add 500 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at maximum speed (≥12000 × g) for 3 minutes.

    Optional: If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
12. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
13. Elute genomic DNA by adding 200 µl of Elution Buffer to the centre of the SciPhi™ Genomic DNA Purification Column membrane. Incubate for 2 minutes at room temperature and centrifuge at 8000 × g for 1 minute.

    Note:

    - For maximum DNA yield, repeat the elution step with additional 200 µl of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material, the volume of the Elution Buffer added to the column can be reduced to 50-100 µl. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
14. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

DNA Isolation from Buccal Swabs

1. Scrape the swab 5-6 times against the inside cheek to collect the sample.
2. Swirl the swab in 200 µl of 1 × PBS for 30-60 seconds.
3. Proceed from step 1 of the Blood DNA isolation protocol (Page No. 8).

TROUBLESHOOTING

Problem	Possible Cause	Solution
Low yield of purified DNA	Excess sample used during lysate preparation	Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocols
	Starting material was not completely digested	Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain
	Ethanol was not added to the lysate	After the addition of ethanol to the lysate mix the sample by vortexing or pipetting
	Ethanol was not added to Wash Buffers.	Make sure that ethanol was added to Wash Buffer I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation on page no. 4
Purified DNA is degraded	Sample was frozen and thawed repeatedly	Avoid repeated freeze / thaw cycles of the samples. Use a new sample for DNA isolation. Perform extractions from fresh material when possible.
	Inappropriate sample storage conditions	Store mammalian tissues at -70 °C and bacteria at -20 °C until use. Whole blood can be stored at 4 °C for no longer than 1-2 days. For long term storage blood samples should be aliquoted in 200 µl portions and stored at -20 °C.
RNA Contamination	RNase A treatment was not carried out	Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	Excess sample was used during lysate preparation.	Reduce the amount of starting material. A maximum of 2×109 of bacteria cells, 5x106 of suspension cells and 20 mg of mammalian tissue is recommended for lysate preparation.
	Tissue was not completely digested	Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain
Inhibition of downstream enzymatic reactions	Purified DNA contains residual ethanol.	If residual solution is seen in the purification column after washing the column with Wash Buffer II, empty the collection tube and re-spin the column for an additional 1 min. at maximum speed (≥12000 × g).
	Purified DNA contains residual salt	Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer I first and then proceed to washing with Wash Buffer II.

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