PRODUCT INFORMATION

Gel Extraction Kit

#NXG994

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Read Storage and Reagent preparation information before first use.

For Research Use Only. Not recommended for diagnostic use.

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Kit Components:

Component	Quantity
Purification Columns with Collection Tubes	50
Binding Buffer	30 ml
Wash Buffer (Conc.)	9 ml
Elution Buffer	15 ml
Ethanol	50 ml

Storage Instructions:

SciPhi™ Gel Extraction kit can be stored at room temperature (15 to 25°C). However, the columns can be stored at 4°C in case of storage periods is more than 1 year,
Please remember to properly close the bag containing SciPhi™ Genomic DNA Purification Columns after each usage.

Product Description:

SciPhi™ Gel Extraction kit is suitable for fast and efficient DNA purification from low-melting and standard agarose, run either in TBE or TAE. This kit is based on spin column silica membrane technology. This kit allows you to purify DNA fragments within a size range of 25 bp to 20 kb. With remarkable recovery rates of up to 95% for DNA fragments sized between 100 bp and 10 kb, it guarantees high-quality results. Each SciPhi™ purification column has a binding capacity of up to 25 µg of DNA and can process up to 1 g of agarose gel. It can purify DNA in less than 15 minutes. Purified DNA from the kit is ideal for PCR, restriction digestion, ligation and sequencing.

Principle:

Excision: The DNA fragment of interest is first visualized on an agarose gel is carefully cut out from the gel using a clean, sharp tool, like a razor blade or a specialized gel extraction tool.

Solubilization: The gel slice is then solubilized in a binding buffer containing a chaotropic agent. Chaotropic agents disrupt hydrogen bonding, thereby denaturing proteins and dissolving the agarose gel.

DNA Binding: The binding buffer used for solubilization promotes binding of DNA to silica membrane of the column, while other impurities, such as proteins, nucleotides, and unincorporated primers, do not efficiently bind and remain in the solution.

pH Indicator: The binding buffer is often designed with a colour indicator that changes colour with pH. This pH indicator allows easy monitoring of the solution's pH, ensuring that the optimal conditions for DNA binding to the silica are maintained.

Washing: After the DNA binds to the silica, the impurities are washed away from the column using a series of wash steps. These wash steps help to remove any remaining contaminants that might interfere with downstream applications.

Elution: Finally, the purified DNA is eluted from the silica column using an elution buffer, typically with a lower pH than the binding buffer. This elution process helps to release the DNA from the silica and make it available in a clean, concentrated form for downstream applications.

Important Information:

- Prepare **Wash Buffer** according to Table 1 before first use:

  Table 1: Instruction to prepare wash buffers

	Wash Buffer I
Concentrated Wash Buffer	9 ml
Ethanol (96-100%)	45 ml
Total Volume	**54 ml**

  Mark the ethanol added check box printed on bottle stickers after adding ethanol.

- Always check for **salt precipitation** in the Binding Buffer before each use. Warm the solution to 37°C and then cool to 25°C before using to dissolve any precipitate.
- Handle Binding Buffer after **wearing the gloves** as this buffer contains irritants.
- Use **fresh TAE/TBE** buffer when performing electrophoresis on PCR amplicons intended for sequencing.
- Carry out all purification steps at **Room Temperature** only.
- Carry out centrifugation steps in a table-top microcentrifuge at an rcf of **>12000 x g** i.e. approximately equals to 10000-14000 rpm depending on the rotor type.

Additional Instruments and Consumables Required

- Pipettes and pipette tips
- Vortex Mixture
- Microcentrifuge
- Water Bath or Heat Block
- Gloves
- 1.5 ml Microcentrifuge tubes
- Isopropanol
- 3 M Sodium acetate, pH 5.2

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PROTOCOLS FOR PURIFICATION

A. Protocol for Gel Extraction by using a centrifuge

1. Using a clean scalpel or razor blade, cut out the gel slice containing the DNA fragment. Cut as near to the DNA as feasible to reduce gel volume. Place the gel slice in a pre-weighed 1.5 mL tube and weigh it again. Make a note of the weight of the gel slice.

   Caution: Avoid exposing the purified fragment to UV light if it will be utilized in cloning procedures. Reduce UV exposure to a few seconds or keep the gel slice on a glass or plastic plate during UV exposure.

2. Add Binding Buffer in a volume-to-weight ratio of 1:1 to the gel slice (for example, 100 µl of Binding Buffer for every 100 mg of agarose gel).
   Caution: Add 2:1 volumes of Binding Buffer to the gel slice for gels with an agarose concentration greater than 2%.

3. Incubate the gel mixture for **10 minutes** at **50-60 °C**, or until the gel slice has been fully dissolved. To speed up the process, invert the tube after every few minutes for mixing of the suspension. Make sure all of the gel has been dissolved. Briefly vortex the tube before transferring the gel suspension onto the column.

   Examine the colour of the solution. The yellow colour of the solution shows optimal pH for DNA binding. If the solution is orange or violet in colour, add 10 µl of 3 M sodium acetate solution, pH of 5.2. The mixture will turn yellow in hue.

4. **Optional:** Perform this step when DNA fragment is ≤500 bp or ≥10 kb in length.
   a. **When DNA fragment is ≤500 bp:** Add 1 gel volume of 100% isopropanol to the solubilized gel solution (for example, 100 µl of isopropanol should be added to a 100 µl solution of binding buffer containing 100 mg of gel slice). Mix well.
   b. **When DNA fragment is ≥10 kb:** Add 1 gel volume of sterile molecular biology grade water to the solubilized gel solution (for example, 100 µl of water should be added to a 100 µl solution of binding buffer containing 100 mg of gel slice). Mix well.

5. Transfer up to 800 µl of the gel suspension to a SciPhi™ Purification Column placed in a collection tube. Centrifuge the column for 1 minute. Discard the flow-through solution and place the column into the same collection tube.

   Note:

   - Remember to tightly seal the bag of SciPhi™ Purification Columns tightly after each use!
   - The remaining volume of the suspension after transferring 800ul can be transferred to the same column after the first centrifuge. Centrifuge the column again and perform this step until whole suspension is subjected to the column.

6. **Optional:** Perform this step when DNA fragment need to be use for sequencing.

   Add 100 µl of Binding Buffer to the column again and centrifuge for 1 minute. Discard the flow-through.

7. Add 700 µl of Wash Buffer I (with ethanol added) and Centrifuge for 1 minute. Discard the flow-through and place the purification column back into the collection tube.

8. If residual wash buffer remains in the purification column, re-spin the column for 1 minute at maximum speed.

   Note: This step is necessary as residual ethanol if eluted with DNA may inhibit downstream enzymatic reactions.

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 DNA by adding 50 µl of Elution Buffer to the centre of the SciPhi™ Purification Column membrane and centrifuge for 1 minute.

Note

- for low amount of initial DNA, volume of elution buffer can be reduced to 20-50 µl for maximum DNA yield. A volume of less than 10 µl is not recommended for elution.
- Prewarm the elution buffer at 65°C, if DNA fragment size is ≥ 10 kb.
- If the DNA amount is ≤5 µg and elution volume is 10 µl, Incubation of the column for 1 minute is recommended before centrifugation.

11. Discard the purification column and use the purified DNA immediately in downstream applications or store at -20 °C.

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B. Protocol for Gel Extraction by using a vacuum manifold

1. Using a clean scalpel or razor blade, cut out the gel slice containing the DNA fragment and process it according to the step no. 1-4 given on page no. 4.
2. Setup the vacuum manifold according to the manufacturer's instructions and place the purification column(s) onto the manifold.
3. Transfer up to 800 µl of the gel suspension that has been solubilized (from steps 3 or 4 in Protocol A), to the purification column.

   Note:

   - **Remember to tightly seal the bag of SciPhi™ Purification Columns tightly after each use!**
   - **If the suspension solution is ≥ 800 µl, transfer the remaining suspension to the same column and apply the vacuum again and perform this step until whole suspension is subjected to the column.**
4. Apply vacuum pressure to gently draw the suspension through the column. Once the entire sample has passed through, ensure to switch off the vacuum immediately.
5. **Optional:** Perform this step when DNA fragment need to be use for sequencing.

   Add 100 µl of Binding Buffer to the column again and apply vacuum pressure to gently draw the suspension through the column. Once the entire sample has passed through, switch off the vacuum.
6. Add 700 µl of Wash Buffer I (with ethanol added) and apply vacuum pressure to gently draw the suspension through the column and place the purification column back into the collection tube.
7. If residual wash buffer remains in the purification column, centrifuge the column for 1 minute at maximum speed.

   Note: This step is necessary as residual ethanol if eluted with DNA may inhibit downstream enzymatic reactions.
8. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
9. Elute DNA by adding 50 µl of Elution Buffer to the centre of the SciPhi™ Purification Column membrane and centrifuge for 1 minute.

   Note:

   - for low amount of initial DNA, volume of elution buffer can be reduced to 20-50 µl for maximum DNA yield. A volume of less than 10ul is not recommended for elution.
   - Prewarm the elution buffer at 65°C, if DNA fragment size is ≥ 10 kb.
   - If the DNA amount is ≤5 µg and elution volume is 10 µl, Incubation of the column for 1 minute is recommended before centrifugation.
10. Discard the purification column and use the purified DNA immediately in downstream applications or store at -20 °C.

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TROUBLESHOOTING

Problem	Possible Cause	Solution
Low yield of purified DNA	Incomplete solubilization of the gel slice	• Ensure addition of 1:1 volume of Binding Buffer to the precisely weighed gel slice. For example, for every 100 mg of agarose gel, add 100 µL of Binding Buffer. • Ensure complete solubilization of the gel slice before subjecting the suspension to the column. • Take note that a large amount of agarose or a gel slice with an agarose percentage greater than 2% may require additional time to dissolve. If needed, use larger volumes of Binding Buffer and consider additional vortexing of the gel solution to facilitate solubilization.
	Inefficient DNA binding	• After dissolving the gel slice, observe the colour of the solution. A yellow colour indicates an optimal pH for DNA binding. • If the solution colour appears orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution, and mix. The colour of the mix should turn yellow, indicating the optimal pH for DNA binding.
	Inefficient membrane wash	Prior to the first use, ensure that the recommended volume of ethanol was added to the concentrated Wash Buffer (Page No. 5) to guarantee efficient membrane wash during the purification process.
	Inefficient DNA elution	• When eluting the DNA, add the Elution Buffer directly to the center of the membrane, avoiding the sides of the purification column. • Use an elution volume of 20-50 µL and make sure that the volume sufficiently covers the surface of the membrane for effective DNA elution. • For larger amounts of DNA (e.g., >15 µg), either increase the Elution Buffer volume twice or perform two elution cycles to ensure successful purification. • During step 8, make sure to remove all residual wash buffer from the membrane. If necessary, consider a longer centrifugation time (an extra minute) to aid in the removal of the wash buffer.
DNA does not remain in an agarose gel well.	Presence of residual ethanol	Make sure to remove all residual wash buffer from the membrane while performing step 8. Consider a longer centrifugation time to aid in the removal of the wash buffer.
Low-quality sequencing results	Electrophoresis buffer was reused	Use freshly prepared electrophoresis buffers for both gel preparation and gel running when the extracted DNA will be used directly for sequencing.
Unsuccessful downstream applications	Presence of residual ethanol	Make sure to remove all residual wash buffer from the membrane while performing step 8. Consider a longer centrifugation time to aid in the removal of the wash buffer.

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	Inefficient membrane wash	If the collection tube is overfilled during the wash step, some of the wash buffer may not be able to flow through the column entirely and could remain in the column. This can lead to a lower purity of the final eluted nucleic acids and may affect downstream applications. To avoid this, it's essential to always discard the flow-through after centrifugation.
	Eluate contaminated with agarose	Ensure complete solubilization of the gel slice before subjecting the suspension to the column. Ensure addition of 1:1 volume of Binding Buffer to the precisely weighed gel slice. Take note that a large amount of agarose or a gel slice with an agarose percentage greater than 2% may require additional time to dissolve. If needed, use larger volumes of Binding Buffer and consider additional vortexing of the gel solution to facilitate solubilization.
	Eluate contaminated with excess salt	Ensure that the wash in step 7 of Protocol A was effective. Allow the purification column to incubate with the Wash Buffer for several minutes before proceeding to centrifugation

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