PRODUCT INFORMATION

SciPhi™ PCR Purification Kit

#NXG995-L (250 Preps)

#NXG995-M (50 Preps)

#NXG995-S (10 Preps)

#NXG995-XS (5 Preps)

Read Storage and Reagent preparation information before first use.

For Research Use Only. Not recommended for diagnostic use.

Kit Components:

Components	Quantity			
	NXG995-L	NXG995-M	NXG995-S	NXG995-XS
Purification Columns with Collection Tubes	5 x 50	50	10	5
Binding Buffer	5 x 30 ml	30 ml	6 ml	3 ml
Wash Buffer (Conc.)	5 x 9 ml	9 ml	1.8 ml	0.9 ml
Elution Buffer	5 x 15 ml	15 ml	3 ml	1.5 ml

Storage Instructions:

SciPhi™ PCR Purification 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™ Purification Columns after each usage.

Product Description:

SciPhi™ PCR Purification kit is suitable for fast and efficient DNA purification from PCR or ither enzymatic reaction mixtures. This kit is based on spin column silica membrane technology. This Kit swiftly eliminates primers, dNTPs, unincorporated labelled nucleotides, enzymes, and salts from PCR and other reaction mixtures, ensuring high purity. It supports DNA fragment purification ranging from 25 bp to 20 kb with excellent recovery rates of 90-100%. Each column binds up to 25 µg of DNA, facilitating quick procedures that complete in just 5 minutes. Purified DNA is suitable for a variety of downstream applications including sequencing, restriction digestion, labelling, ligation, cloning, *in vitro* transcription, blotting, and *in situ* hybridization.

Principle:

DNA Binding: The binding buffer used for DNA binding 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.
- Carry out all purification steps at **Room Temperature** only.
- Carry out centrifugation steps in a table-top microcentrifuge at rcf of **>12000 x g** *i.e.* approx 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
- Ethanol

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PROTOCOLS FOR PURIFICATION

A. Protocol for DNA purification by using a centrifuge

1. Add an equal volume of Binding Buffer to the completed PCR mixture (e.g., for 100 µl of reaction mixture, add 100 µl of Binding Buffer) in 1:1 ratio and mix well. Ensure the solution turns yellow, which indicates the pH is optimal for DNA binding. If the solution appears orange or violet, adjust the pH by adding 10 µl of 3 M sodium acetate (pH 5.2) and mix again until the solution turns yellow.

   Optional: For DNA fragments ≤500 bp, add an equal volume of 100% isopropanol (e.g., add 100 µl of isopropanol to 100 µl of the PCR mixture combined with 100 µl of Binding Buffer). Mix thoroughly to ensure proper binding.

   Caution: If PCR mixture contains primer-dimers, purification without isopropanol is recommended. However, the yield of the target DNA fragment will be lower

2. Transfer up to 800 µl of the solution 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.

3. Add 700 µl of Wash Buffer (with ethanol added) and Centrifuge for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
4. 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.

5. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
6. 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.

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

B. Protocol for DNA purification by using a vacuum manifold

1. Setup the vacuum manifold according to the manufacturer's instructions and place the purification column(s) onto the manifold.
2. Transfer up to 800 µl of the solution (from steps 1 of 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.**
3. 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.
4. Add 700 µl of Wash Buffer (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.
5. 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.
6. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
7. 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.
8. Discard the purification column and use the purified DNA immediately in downstream applications or store at -20 °C.

TROUBLESHOOTING

Problem	Possible Cause	Solution
Low yield of purified DNA	Inefficient DNA binding	• 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 centre 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.
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.
	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 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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