



SPRIworks Nebulizer Kit

REF A84871

Intended Use

For Research Use Only. Not for use in diagnostic procedures.

The SPRIworks Nebulizer is used for the fragmentation of DNA.

Summary and Explanation

The SPRIworks Nebulizer is used to shear large DNA molecules into fragments of 200 to 2000 bp. Fragments within this range can be used for downstream molecular biology applications, including the SPRIworks Fragment Library Systems.

Principles of the Procedure

Nebulization creates a fine mist by forcing the input DNA through a small hole in the nebulizer unit. The size of the fragments generated by the nebulization process is dependent upon the pressure of the gas supplied by the nebulizer, the viscosity of the solution, and the temperature.

Product Information

SPRIworks Nebulizer Kit

REF No. A84871

Kit contains 10 SPRIworks Nebulizers.

Specifications:

- Some assembly required.
 - Single use only.
 - If the nebulizer components or packaging are damaged, discard the nebulizer.
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Warnings and Precautions

- For Research Use Only.
 - Use appropriate precautions to prevent the inhalation of aerosolized solution.
 - Nebulization of DNA materials that may contain pathogenic viral DNA must be performed in an externally-vented biosafety hood.
 - Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
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Input DNA and Sample Quality Assessment

Beckman Coulter recommends nebulizing 3 to 5 μg of input DNA for use on the SPRIworks Library Systems. Other downstream applications may have different input recommendations.

Ensure that input DNA is not contaminated. Any contamination in the input DNA will carry contamination into the nebulized DNA and downstream applications. Observe local laboratory standard operating procedures for handling, processing, and storing samples. Beckman Coulter recommends that input DNA have the following characteristics:

- DNA is from the target organism and contains no contaminating DNA.
 - DNA is double-stranded for downstream applications requiring double-stranded DNA.
 - DNA is in fragments no smaller than 1.5 kb.
 - DNA is free of particulate matter.
 - DNA has an OD₂₆₀/OD₂₈₀ ratio of approximately 1.8.
 - DNA has a minimum concentration of 50 ng/ μL in water, or 10 mM TRIS (pH 7.4 to 8.0)
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Materials Provided

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Materials Required But Not Provided

- Compressed Nitrogen: Commercial research grade 5.0.
- Dual-Stage Regulator: Commercial dual-stage regulator and compatible tubing.
- Nebulization Buffer: Commercial nebulization buffer or SPRIworks Nebulization Buffer, provided with the SPRIworks Fragment Library Systems.
- TE Buffer: Commercial TE buffer (10 mM TRIS, 1 mM EDTA).

Optional Materials

AMPure XP

Procedure

Verifying the Nebulizer Assembly

The SPRIworks Nebulizer includes the components shown. Prior to using the nebulizer, verify that the nebulizer is correctly assembled. The nebulizer components must be firmly connected for the nebulizer to function as described.

1. Verify that the rubber stopper is securely connected to the nebulizer top. Gently press the rubber stopper into the nebulizer top if the stopper is loose.
2. Verify that the syringe filter is securely inserted into the hole in the rubber stopper. Gently press the syringe filter into the hole in the rubber stopper if the syringe filter is loose.
3. Verify that the aspiration tube is fully inserted into the silicon tubing. Push the silicon tubing up around the base of the aspiration tube, but do not rotate the aspiration tube.
4. Place the assembled nebulizer top on the bench, with the aspiration tube pointing upward. Do not allow the internal surfaces of the nebulizer top to come into contact with any potentially contaminated surfaces.

Nebulizing DNA

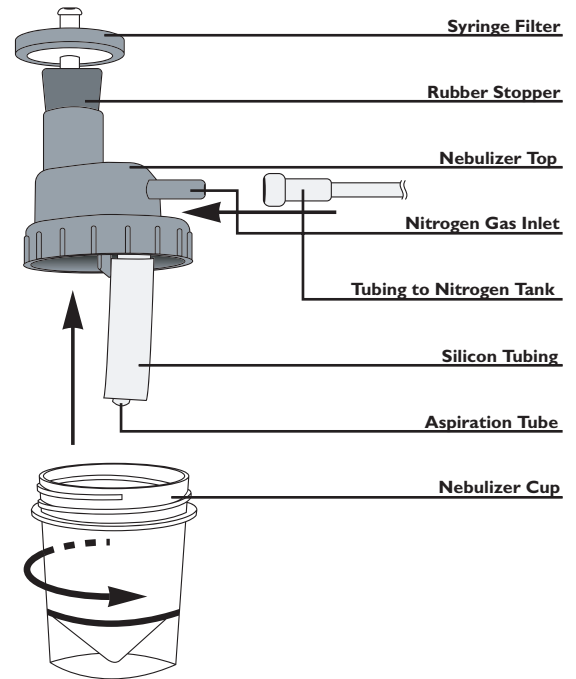
Follow the recommended method for your fragment library construction kit or other downstream application. This procedure requires the use of the SPRIworks Nebulizer and the SPRIworks Nebulization Buffer. This procedure is intended for the fragmentation of input DNA to be used with the SPRIworks Fragment Library System I.

CAUTION: Use the nebulizer in an externally-vented biosafety hood to prevent nebulized DNA from contaminating the air.

1. Obtain an appropriate quantity of input DNA. Use 3 to 5 μg of input DNA for SPRIworks Fragment Library System.
2. Dilute the sample to 100 μL total with 1X TE buffer.
3. Pipette the 100 μL DNA sample into the bottom of the nebulizer cup.
4. Add 500 μL of SPRIworks Nebulization Buffer to the DNA and mix thoroughly by pipetting up and down 5-10 times until mixture is homogeneous.
5. Place the nebulizer top onto the nebulizer cup. Gently turn the nebulizer top clockwise until it is tight.
6. Firmly connect the nebulizer tubing to the nitrogen gas inlet.
7. Connect the other end of the nebulizer tubing to the nitrogen tank.
8. Open the dual-phase regulator to direct 30 psi (2.1 bar) of nitrogen through the nebulizer for two minutes.
9. Close the dual-phase regulator after two minutes have elapsed.
10. Disconnect the tubing from the nitrogen tank and from the nitrogen gas inlet on the nebulizer.
11. Gently tap the nebulizer assembly on a bench top to allow as much nebulized DNA as possible to collect at the bottom of the cup.
12. Carefully turn the nebulizer top counterclockwise until it is loose.
13. Remove the nebulizer top.
14. Collect the nebulized DNA using a pipette.

CAUTION: Do not collect any material that may have lodged outside the nebulizer. This material may not have been completely fragmented and may not be suitable for downstream applications.

15. Measure the nebulized DNA and verify that greater than 300 μL is recovered.



Nebulized DNA Quality

The nebulized DNA may be purified with AMPure XP. After purification, fragmented DNA may be assessed using a quantitative method for determining fragment size and proportion.

Procedural Comments

- A. Refer to the system documentation for a specific description of installation, start-up, principles of operation, system performance characteristics, general operating instructions, operational limitations and precautions, hazards, maintenance, and troubleshooting.
- B. Nebulize DNA in a well-ventilated area such as a ventilated laboratory hood.

Troubleshooting Guide

Problem	Observation	Recommendations
DNA is larger than the desired size range.	Gas pressure maybe too low.	<ul style="list-style-type: none">• Ensure gas pressure is correct.• Check tubing and connections for leaks.
Poor nebulization.	Volumes in excess of 600 μL may negatively affect nebulization.	Do not use a total input volume of greater than 600 μL .
Nebulization results in poor quality or undesirable size range.	The incorrect ratio of TE to nebulization buffer can negatively affect quality and size range.	Use the recommended ratio of TE to nebulization buffer.

Technical Support

If you have any questions about this information, or for technical assistance regarding SPRIworks products or the SPRI-TE Nucleic Acid Extractor, contact Beckman Coulter.

- In the U.S.A. or Canada, contact Beckman Coulter Technical Support by phone at 800-854-3633, or online at www.beckman.com/customer-support. Before using online support the first time, you will need to register online.
- Outside the U.S.A. and Canada, contact your local technical support representative.

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