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Synovial Fluid Bacterial Genomic DNA Isolation Kit Product Insert

Product # 67900

Introduction

Synovial fluid is secreted into the joint cavity from the inner membrane of synovial joints. Synovial fluid is a plasma ultrafiltrate which contains proteins derived from both the blood plasma and produced by cells within the joint tissues. It lubricates the articulating joints as well as supplies oxygen and nutrients while removing carbon dioxide and metabolic wastes from the chondrocytes in the surrounding cartilage. Septic arthritis is usually caused by bacterial, viral or fungal infections to the synovial fluid. *Staphylococcus aureus* is the most common bacterial infection causing Septic arthritis. Diagnosing Septic arthritis is done through joint fluid (synovial fluid) analysis, blood tests or imaging tests.

Norgen's Synovial Fluid Bacterial Genomic DNA Isolation Kit provides a fast, reliable and simple procedure for isolating the highest quality and the highest quantity of bacterial genomic DNA from various amounts of synovial fluid ranging from 1 mL up to 10 mL. Purification is based on using Norgen's proprietary resin separation matrix. The kit is designed to isolate all Gram +ve and Gram -ve strains. Moreover, the kit allows the user to elute the purified bacterial genomic DNA into a flexible elution volume ranging from 25 µL to 50 µL. The purified bacterial gDNA is eluted in an Elution Buffer that is compatible with all downstream applications including PCR, qPCR, methylation-sensitive PCR, Southern Blot analysis and NGS.

Kit Components

Component	Product # 67900 (50 preps)
Precipitation Solution B	15 mL
Lysis Buffer T	6.5 mL
Solution BX	28 mL
Wash Solution A	38 mL
Elution Buffer B	8 mL
Micro Spin Columns	50
Collection Tubes	50
Elution Tubes (1.7 mL)	50
Product Insert	1

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Swinging bucket centrifuge
- Micropipettors
- 15 mL tubes
- 1x PBS (pH 7.4)
- 96 – 100% ethanol
- Water Bath set to 80ºC

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

Quality Control

In accordance with Norgen's Quality Management System, each lot of Norgen's Synovial Fluid Bacterial Genomic DNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Synovial Fluid Bacterial Genomic DNA Isolation Kit is designed for research purposes only. It is not intended for diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as PDF files online at *www.norgenbiotek.com*.

Solution BX contains guanidine hydrochloride (GnHCl), and should be handled with care. (GnHCl) forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Synovial fluid of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with synovial fluid.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Important Notes

- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.
- The spin columns provided with Norgen's Synovial Fluid Bacterial Genomic DNA Isolation Kit are optimized to be used with benchtop centrifuges and not to be used on a vacuum apparatus
- Most standard benchtop microcentrifuges will accommodate Norgen's Micro Spin Columns.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Prepare a working concentration of the **Lysis Buffer T** by adding 18.5 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated Bacteria Lysis Solution. This will give a final volume of 25 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution A** by adding **90 mL of 96 - 100% ethanol** (provided by the user) to the supplied bottle containing the **38 mL** concentrated

Wash Solution A. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added

- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation.

Procedure for the Purification of Bacterial Genomic DNA from Synovial Fluid

1. Add 25 µL **Precipitation Solution B** to every 1 mL Synovial fluid. Mix well by vortexing for 30 seconds.
2. Incubate the mixture for **5 minutes** at room temperature.
3. After incubation, centrifuge for **3 minutes** at **maximum speed.** Discard completely the supernatant.
4. Resuspend the bacterial pellet in 100 µL **1x PBS (pH 7.4)** (not provided). Mix well by vortexing for 30 seconds (***Note****: The bacterial pellet may not be visible depending on the level of bacterial infection*)
5. Add 455 µL **Lysis Buffer T** (after the addition of Ethanol) to the resuspended pellet**.** Mix well by vortexing for 30 seconds.
6. Incubate the mixture at 80ºC for 10 minutes.
7. After incubation, add 500 µL of **Solution BX,** and mix well by vortexing for 10 seconds
8. Add 500 µL of **96-100% Ethanol,** and mix well by vortexing for 10 seconds
9. Transfer the 500 µL of the mixture from **Step 8** into a Micro Spin column assembled with one of the provided collection tubes. Centrifuge for **1 minute at 5,800 x g (~8,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
10. Repeat **Step 9** to transfer the remaining mixture from **Step 8**.
11. Apply 400 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 5,800 x g (~8,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
12. Repeat **Step 11** two more times for a total of 3 washes.
13. Spin the column, empty, for **2 minutes at 13,000 *x g* (~14,000 RPM)**. Discard the collection tube.
14. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 25 - 50 µL of **Elution Buffer B** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.
    - ***Bacterial Genomic DNA from Synovial Fluid is ready for the downstream application of your choice.***

Frequently Asked Questions

1. What if a variable speed centrifuge is not available and the speed differs from the recommended?

- A fixed speed centrifuge can be used, however reduced yields may be observed.

2. At what temperature should I centrifuge my samples?

- All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.

3. What if I added more or less of the specified reagents' volume?

- Adding more or less than the specified volumes may reduce both the quality and the quantity of the purified DNA. Eluting your DNA in high volumes will increase the yield but will lower the concentration. Eluting in small volumes will increase the concentration but will lower the overall yield.

4. What If I forgot to do a dry spin before my final elution step?

- Your purified DNA will be contaminated with the Wash Solution A. This may reduce the quality of your purified DNA and will interfere with your downstream applications.

5. What if my incubation time varied from what is specified in the product manual?

- Varying the incubation time will result in a reduction in your DNA yields.

6. Why do my samples show very low DNA yield?

- The amount of bacterial infection varies from individual to individual. In order to increase the yield, the amount of sample input could be increased.

7. Why are the A260:280 ratio and the A260:230 ratio of the purified DNA low?

- A260:280 and A260:230 ratios are significantly affected by the amount of DNA. If you sample originally contains very low amount of bacteria, the bacterial genomic DNA will be low which will show a low A260:280 and A260:230. I this case the A260:280 and A260:230 ratios should not be used as an indicator for the quality of the purified DNA.

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Synovial Fluid Bacterial Genomic DNA Isolation Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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