**Supplementary Protocol for Total RNA Isolation from Staphylococcus aureus-Related Bacteria and Hard-to-Lyse Bacterial Species**

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**INTRODUCTION**

*Staphylococcus aureus* is a pathogen that causes an array of diseases including impetigo, toxic shock syndrome, necrotizing pneumonia, endocarditis, and sepsis\(^3\). One important *S. aureus* virulence factor that makes its infections so debilitating is that *S. aureus* is *lysozyme-resistant*\(^4\). *Lysozyme* is an enzyme secreted in human airways, nasal submucosal glands, saliva and tears\(^5\) to protect against bacterial and fungal infections. *Lysozyme* is an important component in the innate immune system, as it lyases both gram positive and gram negative bacteria by breaking down peptidoglycan (the primary component of bacterial cell walls). *S. aureus* has been found to be completely resistant to lysozyme, as it has a modification in its peptidoglycan wall—an O acetylation at the C-6 position of the N-acetyl muramic acid\(^6\). As such, *S. aureus* is particularly hard to lyse, as most bacterial nucleic acid isolation techniques involve the use of lysozyme, which is non-functional in *S. aureus*. Lysostaphin is one antibacterial enzyme capable of lysing *S. aureus*. It cleaves cross-linking pentaglycine bridges, which are abundant in the cell walls of staphylococci, making it very efficient\(^6\). Glass bead-beating techniques have also been found to be useful for lysing *S. aureus*. In fact, bead-beating methods have been found to be a faster and more efficient method of lysing hard-to-lyse organisms, resulting in more consistent, higher yields\(^7\). In this study, we present these two techniques for isolating total RNA from *S. aureus*: an enzymatic technique, utilizing lysostaphin, and one physical technique, involving glass beads.

**MATERIALS AND METHODS**

Total RNA was isolated from *S. aureus* using the Total RNA Purification Kit (Norgen Biotek, Thorold, ON) using the bacteria input protocol, with slight modifications as outlined below. Two methods were employed: an enzymatic method (lysostaphin/lysozyme mix) and a bead-beating method, each using 0.75mL of overnight culture spun down at 14000 rpm for 1 minute, with supernatant decanted.

*S. aureus* Lysis via Lysostaphin/Lysozyme Mix

Pelleted cells were frozen in liquid nitrogen, and resuspended in 100 µL of Lysozyme/Lysostaphin mix. The samples were incubated for either 15 minutes, or 30 minutes, at room temperature or 37°C. The amount of Lysis Solution was increased to 350 µL, and the amount of 95% ethanol was also increased to 220 µL. The binding, wash and elution steps were performed as per manufacturer’s instruction.

*S. aureus* Lysis via Glass Bead-Beating

Pelleted cells were resuspended in 400 µL of Lysis Solution, and the entire lysate was transferred to a bead tube (Norgen Biotek, Product #26550). Tubes were vortexed using a commercially available multi-tube vortexer (Vortex Genie®, Scientific Industries, Bohemia, NY) for 5 minutes. Glass beads were pelleted at 14000 rpm for 30 seconds, and the supernatant was transferred into a microcentrifuge tube. Finally, 50 µL of 95% ethanol was added to every 100 µL of supernatant. The binding, wash and elution steps were performed as per manufacturer’s protocol.

**RESULTS AND DISCUSSION**

Total RNA was isolated from *S. aureus* using Norgen’s Total RNA Kit with a modified protocol for hard-to-lyse samples. Two protocols (enzymatic digestion vs. bead tubes) were used to optimize RNA yield and quality. Purified RNA samples were run on a 1X MOPS, 1.5% formaldehyde-agarose gel to assess yield and quality (Figure 1). The purified RNA samples were then quantified using the NanoVue Plus™ spectrophotometer (GE Healthcare, Baie d’Urfe, QC). The bead-beating method and enzymatic digestion method produced high yields (Figure 2), with the bead-beating method producing a slightly higher overall yield than the enzymatic method.

![Figure 1. Comparison of Yield and Quality of RNA Isolated from S. aureus Using Four Different Methods.](image)

Five microlitres from 50 µL of purified RNA was loaded on a 1X MOPS, 1.5% formaldehyde-agarose gel. The enzyme digestion technique worked much more efficiently when incubation took place at 37°C for 30 minutes (compared to room temperature for 15 or 30 minutes). The enzymatic digestion and glass bead technique worked similarly, capturing high quality RNA, with apparent equal amounts of large and small RNA species.
**CONCLUSIONS**

1. **S. aureus cannot be Lysed Using Lysozyme.** *S. aureus* has a modified peptidoglycan wall, making it lysozyme-resistant. Therefore, *S. aureus*, among other hard-to-lyse bacteria, cannot be subjected to regular bacterial RNA isolation kits as they require specialized lysis steps.

2. **Glass Bead-Beating and Lysostaphin Digestion can be used to Efficiently Lyse S. aureus.** We have demonstrated in this report that both glass bead-beating, as well as lysostaphin enzymatic digestion (for 30 minutes at 37°C) can be used to lyse *S. aureus*, generating high quality RNA with high yields.

3. **Lysostaphin Works Very Efficiently at Lysing S. aureus when Incubated at 37°C.** We have shown that incubation temperature can dramatically affect the yield and quality of RNA isolated from *S. aureus* using Lysostaphin enzymatic digestion.

**REFERENCES**