

# 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. One important *S. aureus* virulence factor that makes its infections so debilitating is that *S. aureus* is lysozymeresistant<sup>2</sup>. Lysozyme is an enzyme secreted in human airways<sup>3</sup>, nasal submucosal glands<sup>4</sup>, saliva and tears<sup>5</sup> to protect against bacterial and fungal infections. Lysozyme is an important component in the innate immune system, as it lyses 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<sup>2</sup>. 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<sup>6</sup>. Glass beadbeating 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<sup>7</sup>. In this study, we present these two techniques for isolating total RNA from *S. aureus*: one 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 beadbeating method, each using 0.75mL of overnight culturespun down at 14000rpm for 1 minute, with supernatant decanted.

### *S. aureus* Lysis via Lysostaphin/Lysozyme Mix

Pelleted cells were frozen in liquid nitrogen, and resuspended in 100  $\mu$ L 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  $\mu$ L, and the amount of 95% ethanol was also increased to 220  $\mu$ L. 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  $\mu$ L 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 14000rpm for 30 seconds, and the supernatant was transferred into a microcentrifuge tube. Finally, 50  $\mu$ L of 95% ethanol was added to every 100  $\mu$ L of supernatant. The binding, wash and elution steps were performed as per manufacturer's protocol.

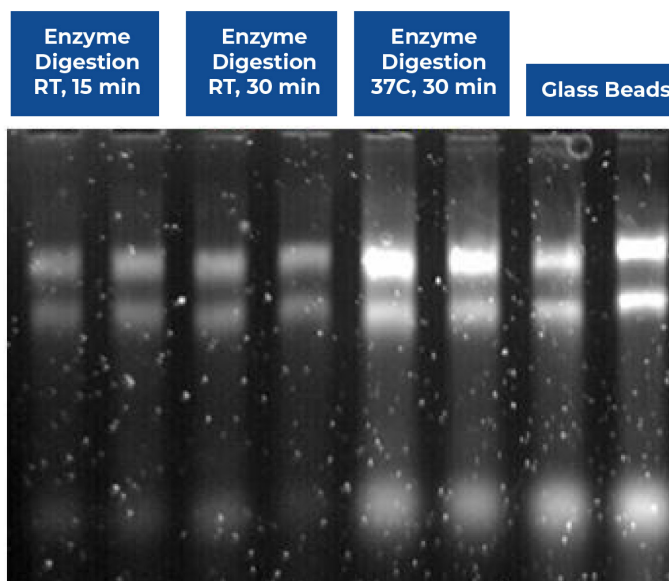
### Agarose Gel Electrophoresis

Purified RNA was then heated to 70°C for 15 minutes with equal volume of RNA loading dye (Norgen Biotek, Thorold, ON), and 5  $\mu$ L of 50  $\mu$ L RNA elutions were loaded on a 1X MOPS, 1.5% Formaldehyde agarose gel. The gel was then run for 30 minutes at 150V.

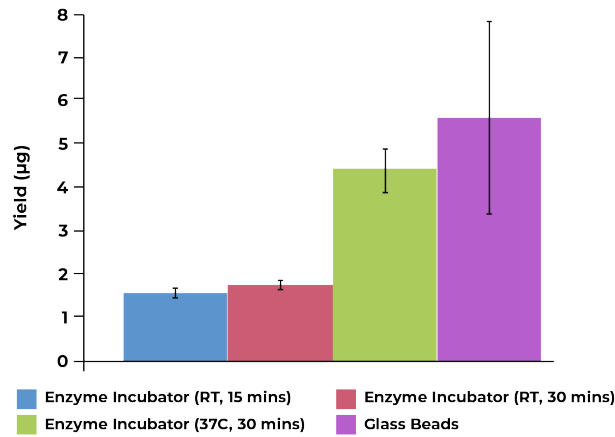
## RESULTS AND DISCUSSION

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% formaldehydeagarose 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 thebead-beating method producing a slightly higher overall yield than the enzymatic method.

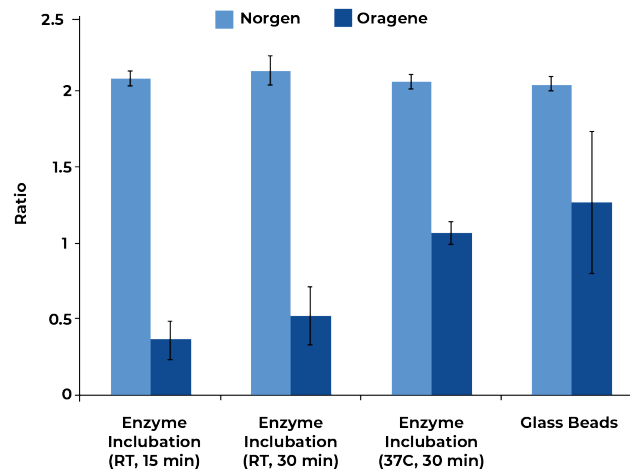
RNA quality was assessed using A260:A280 and A260:A230 ratios generated from the NanoVue Plus™ (Figure 3). Based on A260:A280 ratios, all methods worked similarly (all ratios were between 2.0-2.2). The difference could be found in the A260:A230 ratios, where glass beads and enzyme digestion at 37°C were found to have similar average A260:A230 ratios, which were higher than the enzyme digestion samples incubated at room temperature.



**Figure 1. Comparison of Yield and Quality of RNA Isolated from *S. aureus* Using Four Different Methods.** Five microlitres from 50  $\mu$ L 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.



**Figure 2. Comparison of *S. aureus* RNA Yield from Different Methods Using Nanospectrophotometry.** The purified RNA was quantified using the NanoVue Plus™ (GE Healthcare, Baie d'Urfe, QC). RNA yields were much higher in the enzyme incubation samples when incubation took place at 37°C (compared to room temperature). The enzyme and glass bead techniques generated similar yields, with the glass beads method obtaining the highest average yield.



**Figure 3. Comparison of *S. aureus* RNA Quality from Different Methods Using A260:A280 and A260:A230 Ratios.** RNA quality was also assessed using the NanoVue Plus™ (GE Healthcare, Baie d'Urfe, QC). The enzyme digestion technique as well as the glass bead technique display similar A260:A280 ratios, while the A260:A230 ratios were found to be much higher in the samples incubated at 37°C or lysed via glass beads.

## CONCLUSIONS

1. *S. aureus* cannot be Lysed Using Lysozyme. *S. aureus* has a modified peptidoglycan wall, making it lysozyme-resistant<sup>2</sup>. 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 beadbeating, 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.

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