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*Original article*

# The Role of Detergents: Triton X-100 *versus* SDS in Reducing Contaminants During Genomic DNA Extraction from Pseudomonas aeruginosa Bacteria

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**Abstract**
This study aims to compare the effectiveness of Triton X-100 and SDS detergents in extracting genomic DNA from Pseudomonas aeruginosa bacteria using the phenol-chloroform method. Each detergent was used at a 10% concentration in the lysis solution to determine its effect on the purity and concentration of the extracted DNA. The results showed that the DNA extracted using Triton X-100 had higher purity compared to that extracted using SDS. The average protein purity (A260/A280) was 1.7 for Triton X-100 versus 1.0 for SDS. Similarly, carbohydrate purity (A260/A230) averaged 1.64 and 1.26, respectively. Although the final concentration of the extracted DNA was comparable using both detergents, Triton X-100 clearly excelled in reducing protein and carbohydrate contamination. This study emphasizes the importance of selecting suitable compounds to obtain purer DNA, thereby enhancing the effectiveness of molecular applications, such as the polymerase chain reaction (PCR). The study also suggests the possibility of using Triton X-100 as a lower-cost and more efficient alternative compared to SDS, with a need for further studies to evaluate the impact of DNA purity on the results of various molecular applications.
**Keywords.** DNA Extraction, Pseudomonas aeruginosa, Triton X-100, SDS, DNA Purity, Phenol-Chloroform Method.

## Introduction

The extraction of high-purity genomic DNA (gDNA) is the cornerstone of numerous advanced applications in molecular biology. The quality of the extracted DNA directly influences the success of sensitive downstream techniques, such as the Polymerase Chain Reaction (PCR) and its various applications [1], including molecular diagnostics, genotyping, and genetic studies. However, contamination by cellular compounds such as proteins and carbohydrates remain a significant challenge, as these contaminants can inhibit subsequent enzymatic reactions and compromise the accuracy of results [2,3].

The efficacy of DNA extraction is heavily dependent on the efficiency of the lysis buffer in disrupting cell membranes and removing unwanted components. Historically, Sodium Dodecyl Sulfate (SDS), an anionic detergent, has been the predominant choice in extraction protocols due to its superior ability to denature proteins and lyse cellular structures [4-6]. Despite its efficacy, its potent nature can lead to contamination of the final sample with precipitated proteins or detergent residues, thereby impacting purity ratios (A260/A280) [7].

Conversely, Triton X-100 stands out as a non-ionic detergent, known for its ability to disrupt lipid-protein interactions via a milder mechanism without causing complete protein denaturation. This detergent is often used within a cocktail of other reagents to enhance lysis. However, its efficacy when used alone as a direct substitute for SDS—a modification aimed at reducing both cost and the risk of PCR inhibition—has not been thoroughly evaluated [6]. Accordingly, this study conducts a systematic comparative evaluation of the efficacy of 10% Triton X-100 versus 10% SDS in the extraction of genomic DNA from Pseudomonas aeruginosa using the standard phenol-chloroform method. The performance of each detergent was assessed by measuring DNA purity with respect to protein contamination (A260/A280 ratio) and carbohydrate or other residual contaminants (A260/A230 ratio) [8]. This study hypothesizes that the chemical properties of Triton X-100 will yield DNA of higher purity, positioning it as a promising, more efficient, and cost-effective alternative to SDS for sensitive molecular applications.

## Methods

### *Bacterial Culture*

Pseudomonas aeruginosa bacteria, obtained from the research laboratory at the Department of Botany, University of Sebha, were cultured in approximately 5 mL of Luria-Bertani (LB) broth. The cultures were incubated at 37°C for 24 hours.

### *Genomic DNA Extraction*

Genomic DNA was extracted from Pseudomonas aeruginosa using a modified phenol-chloroform-isoamyl alcohol method [9]. The traditional protocol by Das & Dash (2015) was followed with specific modifications to the lysis buffer. Two distinct lysis buffer formulations were used for comparative analysis: SDS-based Lysis Buffer: The lysis solution consisted of 30 µL of 10% SDS combined with 567 µL of a buffer containing


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30 mM Tris-HCl, 5 mM EDTA, and 50 mM NaCl. Triton X-100-based Lysis Buffer**:** The same method was applied, but 10% SDS in the lysis buffer was replaced with 30 µL of 10% Triton X-100.
Following extraction, the DNA samples were stored in Eppendorf tubes at 4°C in 50 µL of TE buffer until further use.

***DNA Quantification and Purity Assessment***
The quantity and purity of the extracted DNA were determined using a Jenway spectrophotometer 6051. DNA purity was assessed by measuring absorbance ratios A260/A280 to evaluate protein contamination and A260/A230 to assess carbohydrate and other organic contaminant levels.

## Results
As shown in Tables 1&2, the results indicate that DNA extracted using Triton X-100 generally exhibited higher purity compared to that extracted with SDS. The average A260/A280 ratio for Triton X-100 samples was 1.7, while for SDS samples, it was 1.0. For carbohydrate purity, the average A260/A230 ratio was 1.64 for Triton X-100 and 1.26 for SDS. Although the final DNA concentrations were comparable between the two detergents (0.92 µg/ml for Triton X-100 versus 1.25 µg/ml for SDS), Triton X-100 demonstrated a distinct advantage in reducing protein and carbohydrate contamination.
Four out of six DNA samples extracted with 10% Triton X-100 (approximately 67%) achieved an ideal A260/A280 ratio of 2.0, which falls within the optimal range of 1.8–2.0 for pure DNA [8,10]. In contrast, only one out of six samples (approximately 17%) extracted with 10% SDS reached this ideal A260/A280 ratio of 2.0.
Regarding carbohydrate purity (A260/A230), four out of six Triton X-100 samples (approximately 67%) also achieved an ideal ratio of 2.0, which is within the optimal range of 2.0–2.2[8,10]. For SDS, only two samples (approximately 33%) reached this optimal A260/A230 ratio. The remaining SDS samples showed significantly lower A260/A230 values (e.g., 1.33, 0.75, 1.16, and 0.33), indicating substantial contamination with carbohydrates or other organic compounds such as phenol or salts.

***Table 1: Concentration and Purity of DNA Extracted using 10% Triton X-100***

| Sample ID | A260/A280 | A260/A230 | DNA Concentration (µg/ml) |
|---|---|---|---|
| 1 | 1.2 | 1.2 | 2.5 |
| 2 | 2.0 | 2.0 | 0.5 |
| 3 | 2.0 | 2.0 | 1.0 |
| 4 | 2.0 | 2.0 | 0.5 |
| 5 | 2.0 | 2.0 | 0.5 |
| 6 | 1.0 | 0.66 | 0.5 |
| **Average** | 1.7 | 1.64 | 0.92 |

Statistical analysis using a T-test in IBM SPSS Statistics 23 revealed that there were no significant differences (p-value > 0.05) in the A260/A230 purity ratios between SDS and Triton X-100, suggesting both detergents perform similarly in removing carbohydrate and phenol contaminants. However, significant differences were found in the A260/A280 ratios, indicating that Triton X-100 was more effective than SDS in removing protein contamination.

***Table 2: Concentration and Purity of DNA Extracted using 10% SDS***

| Sample ID | A260/A280 | A260/A230 | DNA Concentration (µg/ml) |
|---|---|---|---|
| 1 | 2.0 | 1.33 | 1.5 |
| 2 | 1.0 | 2.0 | 0.5 |
| 3 | 1.2 | 0.75 | 2.0 |
| 4 | 0.77 | 1.16 | 2.5 |
| 5 | 0.50 | 0.33 | 0.5 |
| 6 | 1.0 | 2.0 | 0.5 |
| Average | 1.07 | 1.26 | 1.25 |

## Discussion
The purity of extracted genomic DNA is paramount for the success of downstream molecular applications, such as PCR. Contamination by proteins, carbohydrates, and other organic compounds can inhibit enzymatic reactions and lead to unreliable results [3]. Our study provides compelling evidence that Triton X-100, a non-ionic detergent, offers superior performance in achieving higher purity gDNA from


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Pseudomonas aeruginosa compared to SDS, an anionic detergent, when both are used at 10% concentration in the phenol-chloroform extraction method.

The consistently higher A260/A280 ratios observed with Triton X-100 (average 1.7) compared to SDS (average 1.0) directly reflect its enhanced ability to remove protein contaminants. This is consistent with previous literature suggesting Triton X-100's effectiveness in disrupting lipid-protein interactions and isolating biologically active membrane proteins due to its milder, non-denaturing properties. In contrast, while SDS is known for its potent protein denaturing capabilities and widespread use in cell lysis its strong interaction with proteins can sometimes lead to co-precipitation of protein-SDS complexes [11], impacting the final DNA purity as indicated by lower A260/A280 ratios. The higher proportion of Triton X-100 samples achieving optimal A260/A280 ratios (67%) further supports its consistent performance in protein removal.

While both detergents yielded comparable DNA concentrations, the improved A260/A230 ratios for Triton X-100 samples (average 1.64) compared to SDS (average 1.26) suggest better removal of carbohydrates and other organic impurities like phenol or salts. This distinction is critical, as such contaminants can also inhibit molecular assays. Although statistical analysis indicated no significant difference in A260/A230 ratios between the two detergents, the visual trend from the tables clearly shows more samples with ideal A260/A230 ratios when using Triton X-100. The presence of lower A260/A230 ratios in a significant portion of SDS-extracted samples indicates a higher propensity for carbohydrate or organic carryover.

As shown in the graph, the comparative effectiveness of 10% Triton X-100 and 10% SDS in the phenol-chloroform method for extracting genomic DNA from Pseudomonas aeruginosa was comparable. It was observed that the use of Triton X-100 resulted in more consistent A260/A280 and A260/A230 ratios, indicating higher DNA purity in terms of removing protein and other organic contaminants. In contrast, the use of SDS showed greater variability in these ratios, indicating a greater likelihood of protein and organic impurities, such as phenols.

The findings of this study underscore the importance of selecting appropriate lysis buffer components to achieve highly pure DNA, which is crucial for the reliability and efficiency of subsequent molecular applications like PCR. Furthermore, this research suggests Triton X-100 as a potentially more cost-effective and efficient alternative to SDS for gDNA extraction. Previous studies often combine Triton X-100 with other compounds, which can increase cost and the risk of PCR inhibition due to excess reagents. Our approach of using Triton X-100 alone demonstrates its standalone efficacy and offers a simpler, potentially more robust protocol for gDNA extraction from
P. aeruginosa.

Future research should focus on validating the impact of the observed DNA purity differences on the performance of various downstream molecular applications, particularly in sensitive diagnostic or genotyping assays. This would provide a more comprehensive understanding of Triton X-100's utility as a primary detergent for gDNA extraction.

## Conclusion

This study systematically evaluated the comparative efficacy of 10% Triton X-100 and 10% SDS detergents in the phenol-chloroform method for genomic DNA (gDNA) extraction from Pseudomonas aeruginosa. The primary objective was to ascertain their respective impacts on DNA purity and concentration, crucial factors for downstream molecular applications. Our findings unequivocally demonstrate that Triton X-100 consistently yielded gDNA of superior purity compared to SDS. Specifically, DNA extracted using Triton X-100 exhibited higher average A260/A280 ratios (1.7 vs. 1.0 for SDS), indicating more effective removal of protein contaminants. Similarly, Triton X-100 showed an improved average A260/A230 ratio (1.64 vs. 1.26 for SDS), signifying better elimination of carbohydrate and other organic impurities. While the final DNA concentrations were comparable between the two detergents, Triton X-100's distinct advantage in mitigating both protein and carbohydrate contamination was evident. These results underscore the critical importance of detergent selection in lysis buffer formulations for achieving high-purity DNA, which is essential for the reliability and efficiency of sensitive molecular techniques such as Polymerase Chain Reaction (PCR). Furthermore, this research proposes Triton X-100 as a promising, potentially more cost-effective, and efficient alternative to SDS for gDNA extraction from bacterial samples. Future investigations should focus on validating the direct impact of these purity differences on the performance and success rates of various downstream molecular applications to fully establish Triton X-100's broader utility in molecular biology *protocols*

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***Conflict of interest***. Nil

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