Superior Performance of KACTUS TelN Protelomeras and Optimized Reaction Buffer

Background

TelN protelomerase is an enzyme derived from bacteriophage N15 that cuts dsDNA at the recognition site TelRL and covalently ligates at the cleavage site forming hairpin termini. This unique ability allows it to convert linear plasmid DNA into closed-end DNA (ceDNA) with high efficiency. ceDNA is valuable in gene editing, where small and stable DNA templates of therapeutic genes are needed for knock-in purposes. By adding one recognition site at each end of the gene of interest (GOI), researchers can obtain GOI-only DNA templates, minimizing cytotoxicity compared to using a full-length plasmid as a DNA template. High-performing and reliable TelN Protelomerase is critical for researchers producing high-quality ceDNA.

Experimental Overview

A leading biopharma company conducted a comparative study to evaluate the performance of the KACTUS TelN Protelomerase enzyme against that of a leading supplier. The study aimed to assess both the enzyme's efficiency in producing desired ceDNA and the effectiveness of each supplier's reaction buffers. The experiment used a plasmid containing two TelN recognition sites and was conducted under identical conditions for both enzymes. The plasmid was mixed with TelN and reaction buffer for a total reaction volume of 20 µL. The reaction was incubated for 30 minutes at 30°C, and the results were analyzed using Genomic DNA TapeStation ScreenTape. The customer tested the KACTUS and other supplier's TelN with both the KACTUS optimized reaction buffer and the other supplier's reaction buffer.

Results: Increased ceDNA concentration using KACTUS TelN Protelomerase and optimized buffer

Enzyme Performance: The KACTUS TelN enzyme outperformed the supplier's TelN protelomerase enzyme in ceDNA yield. At a condition of 5 units (U) and 40 ng/µL concentration, the KACTUS TelN enzyme produced a higher concentration of ceDNA compared to the supplier's enzyme, for both reaction buffers.
Buffer Performance: The teal columns in the graph illustrate the increased ceDNA production when the KACTUS buffer was used. Our buffer resulted in higher ceDNA concentrations (teal columns) for all conditions tested compared to the supplier's buffer (yellow columns).

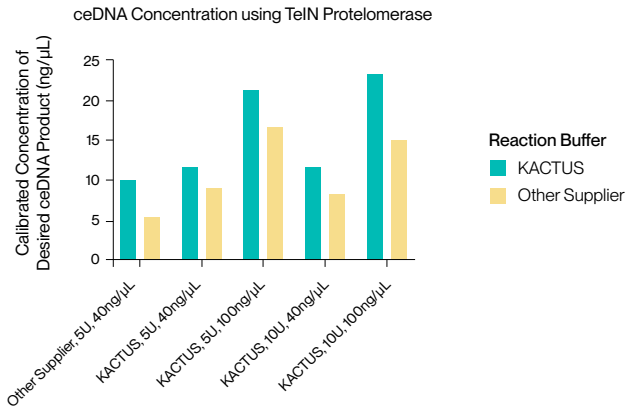

Figure 1. *Comparison of ceDNA concentration using KACTUS and a leading supplier's TelN Protelomerase enzymes with their respective reaction buffers. The graph illustrates ceDNA concentrations using TelN Protelomerase from KACTUS and another supplier. The enzymes are tested at various concentrations with both KACTUS optimized buffer (teal) and the supplier's buffer (yellow). The results highlight the superior efficiency of the KACTUS enzyme and optimized buffer, particularly at higher enzyme concentrations (10U, 100 ng/µL), where the KACTUS combination produces the highest yield of desired ceDNA product.*

Conclusion: This study demonstrates that the KACTUS TelN Protelomerase enzyme, when paired with our optimized reaction buffer, outperforms a leading supplier's enzyme and buffer system during ceDNA production. Researchers looking to enhance their telomerase activity assays and ceDNA yields may benefit from choosing the KACTUS TelN Protelomerase and reaction buffer.