

Reducing DNA content during protein purification with an easily removable nuclease

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Introduction

When purifying DNA-modifying enzymes, an essentially DNA-free product is desirable as endogenous DNA from production may contaminate down-stream applications.

While nuclease treatment of the crude cell-lysate is a common step during protein purification, its primary function is viscosity reduction for easier sample handling rather than complete DNA removal. More efficient enzymatic DNA removal could be achieved if applied to a partially purified protein sample, such as the His-trap eluate, where the majority of impurities have been removed and the volume is reduced; however the salt concentrations used in elution protocols are incompatible with the salinity optima of most commercially available nucleases. Furthermore, treatment of semi-pure protein may cause residual nuclease contamination in the purified protein which will interfere with nucleic-acid based applications.

ArcticZymes has developed a Heat-Labile Salt Active Nuclease (HL-SAN) that is compatible with treatment of eluates: it is active at up to 1M NaCl, tolerates imidazole and can be separated from most proteins by cation exchange or inactivated by reducing agents. Here we present efficient DNA removal from recombinantly-produced T4 DNA ligase by direct treatment of the His-trap eluate with HL-SAN, and demonstrate that the final, active T4 ligase is free of contaminating HL-SAN activity.

Results

HL-SAN reduces DNA in the crude lysate

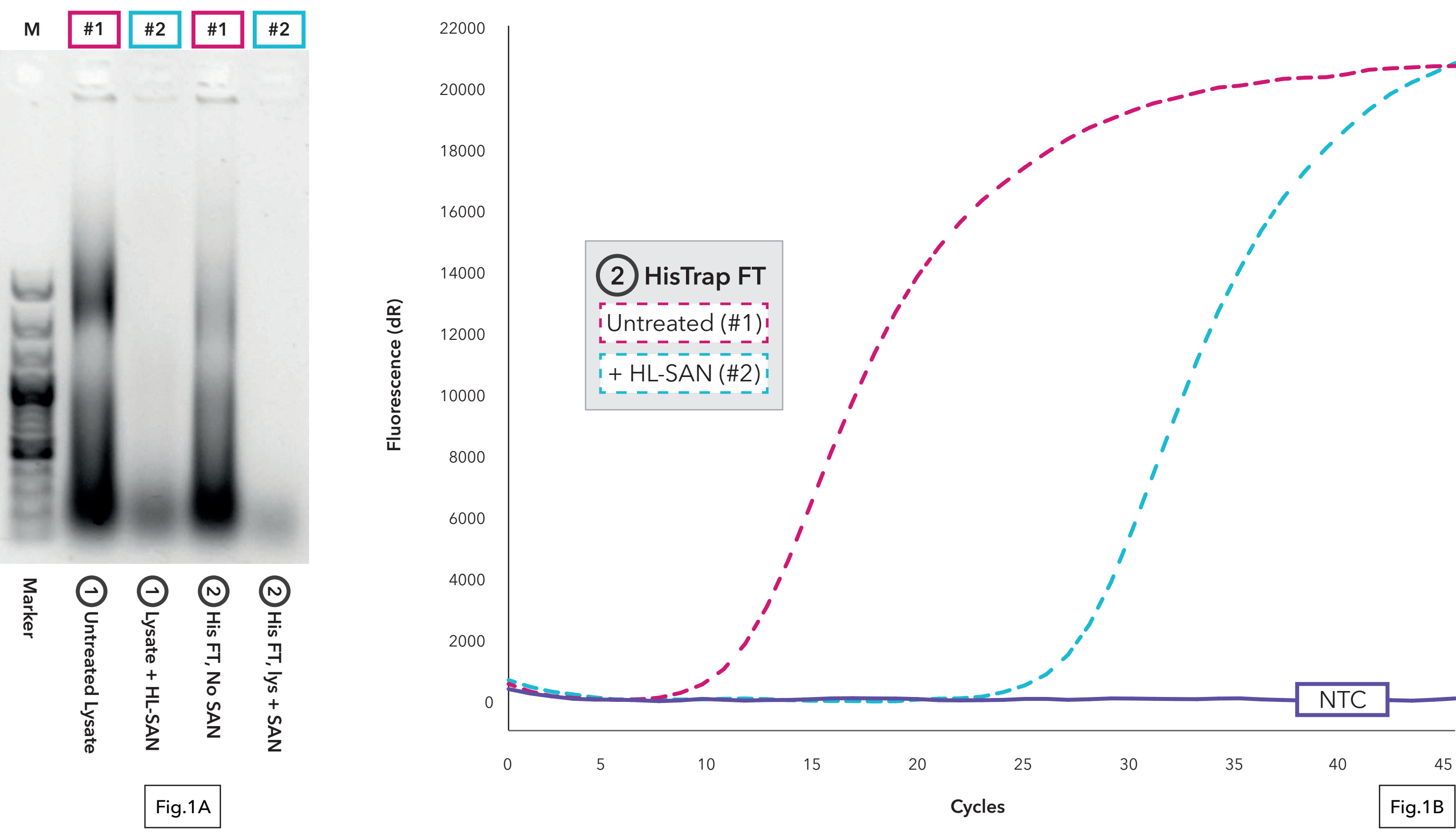


Figure 1A: HL-SAN greatly reduced DNA content of the crude lysate, shown by agarose gel electrophoresis of undiluted lysate and His-trap flow-through, and in both cases the majority of the DNA remained in the flow-through.
Figure 1B: *E. coli* 23S DNA in flow-through was quantified by qPCR, and it was determined that HL-SAN treatment of the lysate reduced DNA content about 10,000 fold.

HL-SAN is removed by cation exchange chromatography

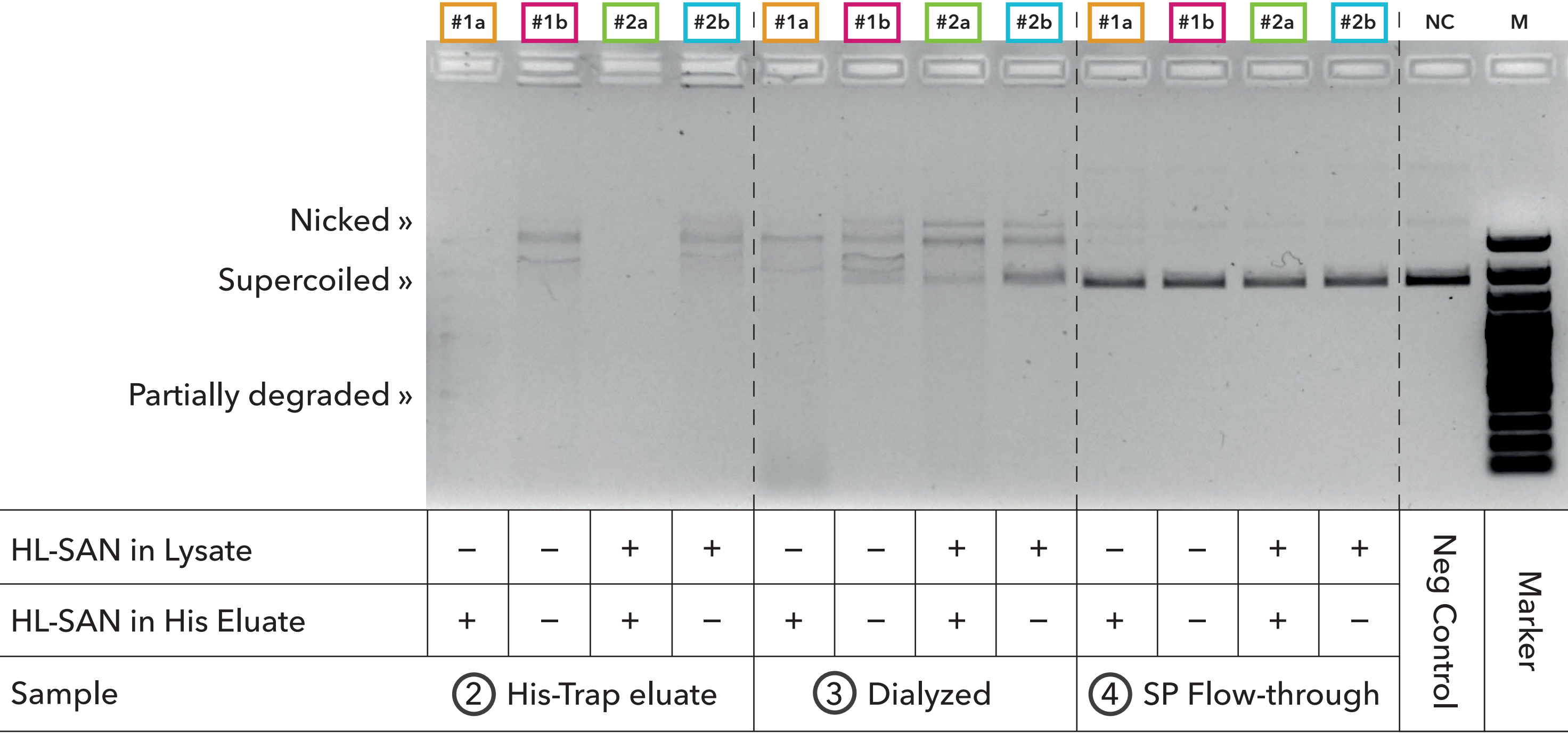
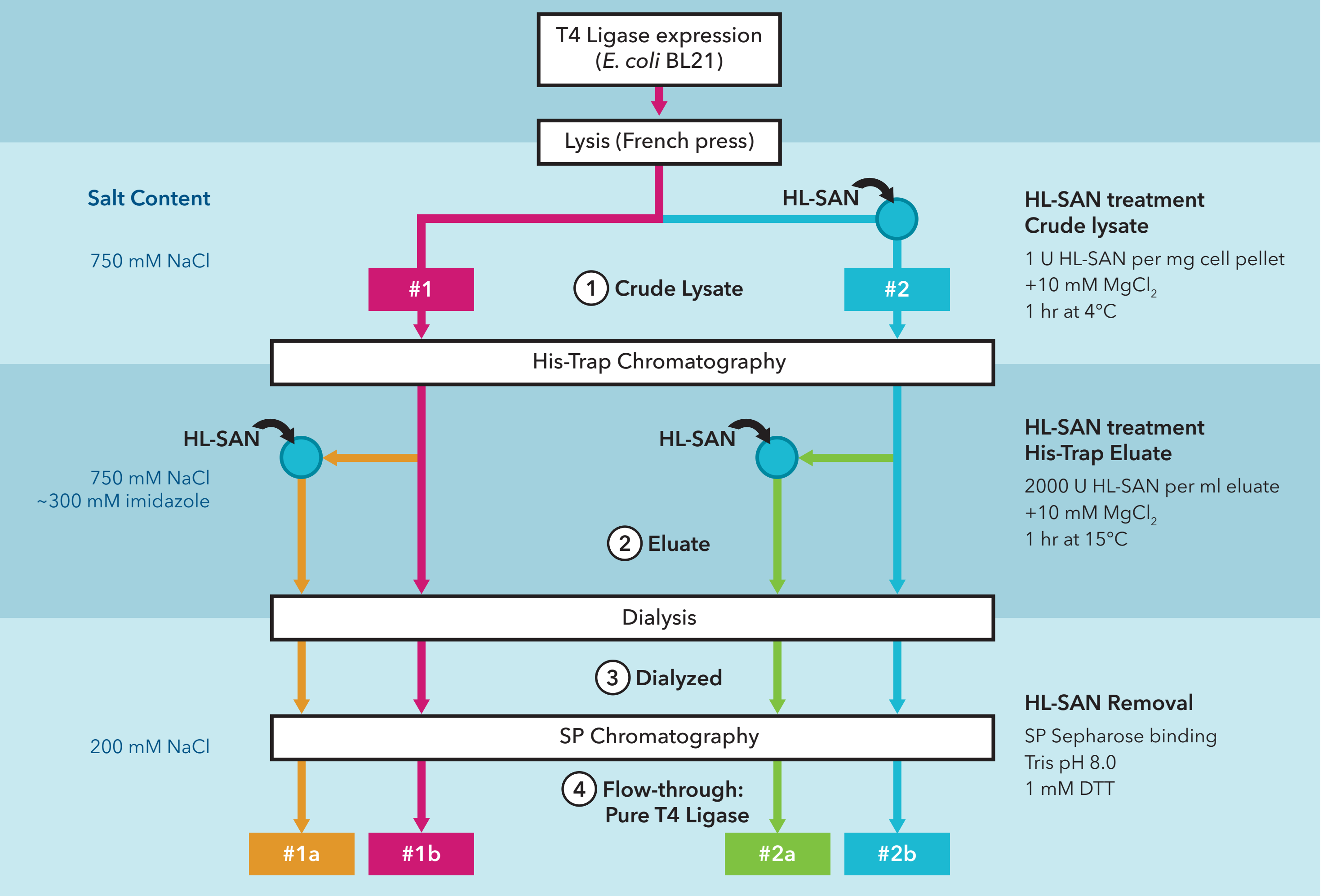


Figure 3: HL-SAN was highly active in His-trap eluate, where 1 µl of sample completely digested 25 µl double stranded plasmid DNA (45 mM Tris pH 8, 20 mM MgCl₂) after 2 hours at 37°C. The majority of this activity was removed by passing eluate over an SP sepharose column, after which only minor DNA nicking was observed, corresponding to the background EndA activity from the BL21 host-cell strain.

Experimental setup



HL-SAN reduces DNA in His-trap eluate

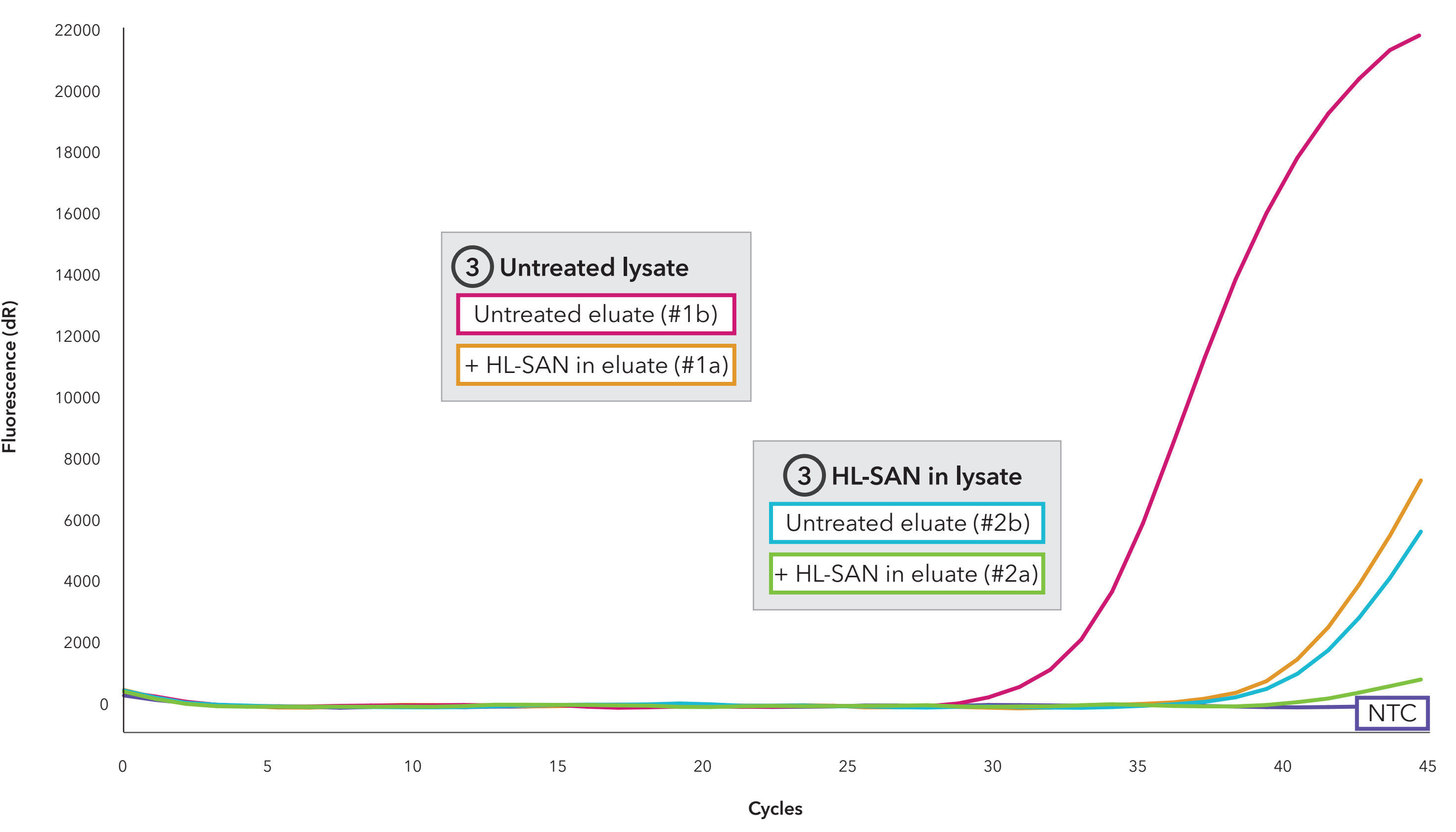


Figure 2: Although most of the DNA was removed by the His-Trap, some is still present in the eluate. HL-SAN treatment of the lysate reduced DNA in the flow-through 10,000 fold, however the reduction in the eluate was only about 200-fold. A similar reduction was achieved by direct HL-SAN treatment of the eluate alone, while combining HL-SAN treatment of both lysate and eluate resulted in about 4,000-fold reduction in DNA as compared to untreated sample.

T4 Ligase is unaffected by HL-SAN treatment



Figure 4: DNA ligase activity was assayed by incubation of 80 nM of a singly-nicked double-stranded substrate with 0.6 µM T4 product for 15 min at 25°C (0.1 mM ATP, 10mM MgCl₂, 1mM DTT 100 mM NaCl, 50 mM Tris pH 8.0). The ligated band (18-bp) and un-ligated bands (9-bp) were resolved by denaturing gel electrophoresis (20% acrylamide, 7M urea, 1x TBE gel) and visualized by excitation of a fluorescent label. No difference in the specific activity of the concentration-normalized T4 ligase was seen with HL-SAN treatment, and no degradation of assay substrates due to residual nuclease activity was detected.

Conclusions

- HL-SAN efficiently digests DNA in high salt His-Trap eluates containing imidazole
- The lowest DNA contamination in the final product is achieved by HL-SAN treatment of both the lysate and the eluate
- HL-SAN is efficiently separated from T4 ligase product by cation exchange chromatography
- T4 ligase activity is unaffected by HL-SAN treatment