ArcticZymes®
Enzyme innovations from the Arctic

HL-SAN for DNA removal in protein purification

Nucleic acids, and especially genomic DNA, often pose a problem in purification of DNA-binding proteins as they interfere with purification, downstream analysis or applications. Nucleases activity is usually difficult to remove while HL-SAN is easily inactivated or separated from other proteins. This enables nuclease treatment without residual nuclease activity in downstream applications.

HL-SAN is easily inactivated by treatment with a reducing agent, and the high pI (9.6) enables easy separation of HL-SAN from a vast majority of protein targets. The optimal activity at high salinity and the resistance to non-ionic detergents enable HL-SAN treatment at conditions facilitating dissociation of DNA from DNA-protein complexes to make it more accessible for degradation. These features make HL-SAN the superior choice for DNA digestion in your protein purification workflow.

Guidelines for DNA removal

The amount of HL-SAN needed for DNA removal from a cell extract or lysate depends on several factors; expression strain, target protein, lysis buffer composition, NaCl concentration, etc. The following is therefore considered as guidelines: Add 1000 U HL-SAN per ml sample with 0.3–0.75 M NaCl and incubate at 15–37°C for 30–60 minutes or at 4°C overnight. Mg is required for activity.

DNA may cause a problem during protein purification and in the final product. In the first steps of a purification scheme, only fragmentation of genomic DNA in the lysate/extract is usually necessary. However, even small amounts of DNA can result in a contaminated product and using HL-SAN in later steps in the protein purification workflow will facilitate removal of traces of nucleic acids (decontamination).

Inactivation

Inactivation is achieved by adding reducing agents like TCEP or DTT, where TCEP* is the recommended reducing agent. The inactivation protocol can be adapted to several workflows by varying incubation time, temperature and concentration of the reducing agent. In general >99% inactivation is achieved after 5–10 minutes at 25–37°C. To avoid reactivation, maintain a low concentration of reducing agent, 0.1–0.5 mM DTT or TCEP, or use prolonged incubation times with 10–20 mM TCEP upon inactivation.

*Low stability in phosphate buffers, especially at neutral pH.

HL-SAN

Easily inactivated

High pI - easy to remove

Optimal activity at high salinity

Recommended operating conditions

Condition	Optimal	Effective*
Salt (NaCl/KCl)	500 mM	50 mM–1 M
Temperature	~35°C	4–50°C
Mg^{2+}/Mn^{2+}	5–20 mM	1–40 mM
pH	9.0	7.0–9.5

***Effective is defined as the condition which HL-SAN has ≥ 10 % of its activity as compared to optimal conditions.**

Guidelines for inactivation

Temperature/Time	DTT	TCEP
4°C/18 hours	-	10 mM
25°C/60 minutes	10 mM	5 mM
30°C/30 minutes	10 mM	5 mM
40°C/30 minutes	5 mM	1 mM
50–70°C/30 minutes	1 mM	1 mM

Removal

The very high pI (9.6) of HL-SAN results in tight binding to cationic columns. Even at pH 9.0 with 0.2M salt, less than 0.02% leakage in flow-through/wash is observed. It is not recommended to use anionic IEX columns for removal of HL-SAN as the glycosylation of HL-SAN result in column binding.

Conditions for binding HL-SAN to SP-sepharose

pH	Salt
pH 7	≤0.3 M
pH 8	≤0.3 M
pH 9	≤0.2 M

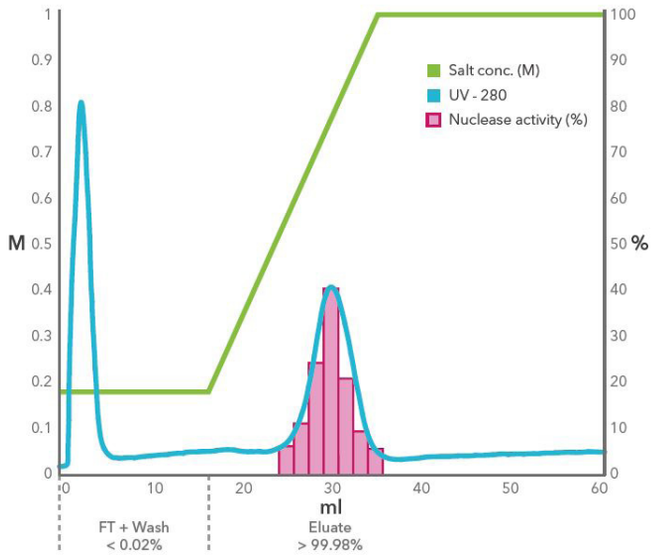

Figure 1: HL-SAN binds tightly to SP-sepharose columns at pH 9.0 with 0.2M salt (less than 0.02% leakage).

DNA removal from various samples

Sample	HL-SAN final concentration		Recommended conditions
	DNA removal*	Decontamination**	
Protein	100 U/ml	1000 U/ml	30 minutes at 25–37°C
Reagent	100 U/ml	1000 U/ml	30 minutes at 25–37°C
Cell extract	1000 U/ml	N/A	60 minutes at 25–37°C or 4°C overnight
Cell lysate (soluble fraction)	500 U/ml	N/A	60 minutes at 25–37°C or 4°C overnight
Viscosity reduction	25–50 U/ml		10–20 minutes at 25°C

*** DNA amount is reduced to a level that cannot be detected by visualization using agarose gel electrophoresis.**
**** DNA amount is reduced to a level generally not detectable by a 23S rDNA qPCR assay.**

Properties

Source	Recombinantly produced in *Pichia pastoris*.
Activity	HL-SAN is highly active in the temperature range 10–50 °C. Optimal NaCl-concentration for activity is 0.5 M, working range is 0.25–1 M. Mg^{2+} (>1 mM) is required for activity. Working pH range is 7.5–9.5, optimal pH is 9.0.
Specific activity	≥ 175 000 Units/mg
Unit definition	One Unit is defined as an increase in absorbance at 260 nm of 1 A in 30 minutes at 37 °C, using 50 µg/ml calf thymus DNA (D-1501, Sigma) in a buffer consisting of 25 mM Tris-HCl, pH 8.5 (25 °C), 5 mM $MgCl_2$, 500 mM NaCl.

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