TECHNICAL NOTES

Salt Active Nuclease

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A Highly Salt-Tolerant Nuclease for Effective DNA Removal in Protein Purification

Salt Active Nuclease for DNA digestion

The unique features make Salt Active Nuclease (SAN) the superior choice for DNA digestion in protein purification schemes, particularly in the purification of DNA-binding proteins and enzymes which must be free of nuclease activity and contaminating DNA.

- Only nuclease with optimum activity in 0.5 M NaCl
- Active at low temperature
- Easily inactivated by reducing agents like DTT
- High pI – easy to remove by ion exchange

How it works

Salt Active Nuclease is a novel, engineered nonspecific endonuclease with optimum activity at high salt concentrations. The size of the end products from ssDNA varies from ~ 5 – 13 nt, while dsDNA is digested to around ~ 5 – 7 nt.
It is active in a variety of buffers and at low temperatures. The enzyme is easily inactivated by treatment with a reducing agent, and the high pI (9.6) enables easy separation from a vast majority of protein targets. Its inactivation properties could also make the enzyme convenient for decontamination of molecular biology reagents.

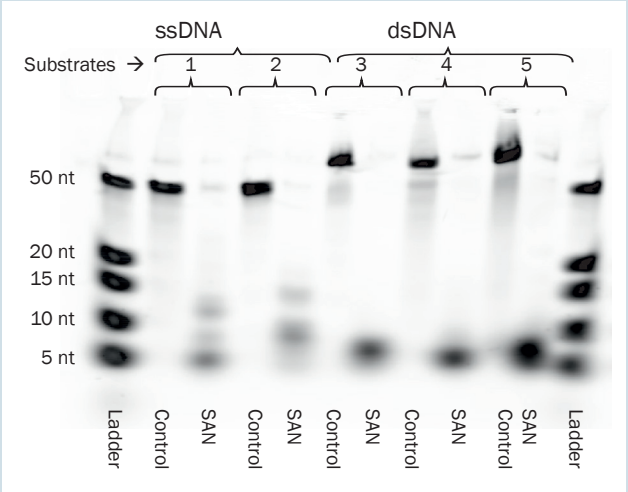

Fig. 1: Salt Active Nuclease (SAN) digests ssDNA to ~ 5 – 13 nt and dsDNA to ~ 5 – 7 nt.

High salt tolerance can improve workflow and yield

Salt is an important component of various purification schemes. The presence of salt can minimize protein or virus aggregation, increase target solubility and improve target yield. High salt enables contaminating DNA to dissociate from associated proteins and become available for degradation. But nuclease like Benzonase®* are inhibited by high concentrations of salt. Salt Active Nuclease, however, is very active even in concentrations as high as 1 M NaCl (fig. 2).

*Benzonase® is a trademark of Merck KGaA.

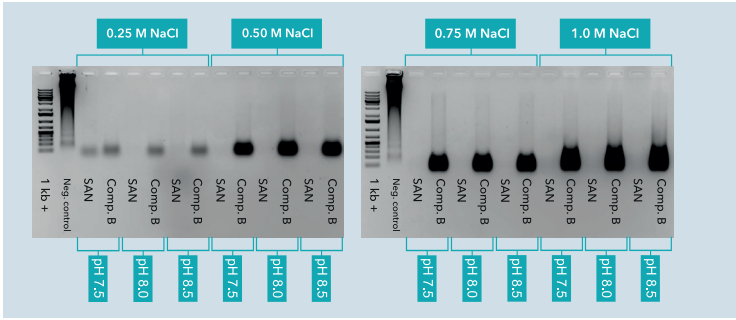

Fig. 2: Salt Acitive Nuclease (SAN) is very active at high concentrations of salt where other commercial nonspecific nucleases are relatively inactive.

Inactivation of SAN

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

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

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Guidelines for DNA removal by SAN

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 Salt Active Nuclease in later steps in the protein purification workflow will facilitate removal of traces of nucleic acids (decontamination).

The amount of Salt Active Nuclease needed for DNA removal from a cell extract or lysate depends on several factors like expression strain, target protein, lysis buffer composition, NaCl concentration, etc. The following is therefore considered as guidelines: Add 1000 U Salt Active Nuclease 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^{2+} is required for activity.

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 in which Salt Active Nuclease has ≥10 % of its activity as compared to optimal conditions.

DNA removal from various samples

Condition	Salt Active Nuclease final concentration		Recommended conditions
	DNA removal*	Decontamination**	
Protein	100 U/ml	1000 U/ml	30 min at 25 – 37 °C
Reagent	100 U/ml	1000 U/ml	30 min at 25 – 37 °C
Cell extract	1000 U/ml	N/A	60 min at 25 – 37 °C or 4 °C overnight
Cell lysate (soluble fraction)	500 U/ml	N/A	60 min at 25 – 37 °C or 4 °C overnight
Viscosity reduction	25 – 50 U/ml		10 – 20 min 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.

Removal of SAN

The very high pI (9.6) of Salt Active Nuclease 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 (fig. 3). It is not recommended to use anionic IEX columns for removal of Salt Active Nuclease as the glycosylation of the enzyme results in column binding.

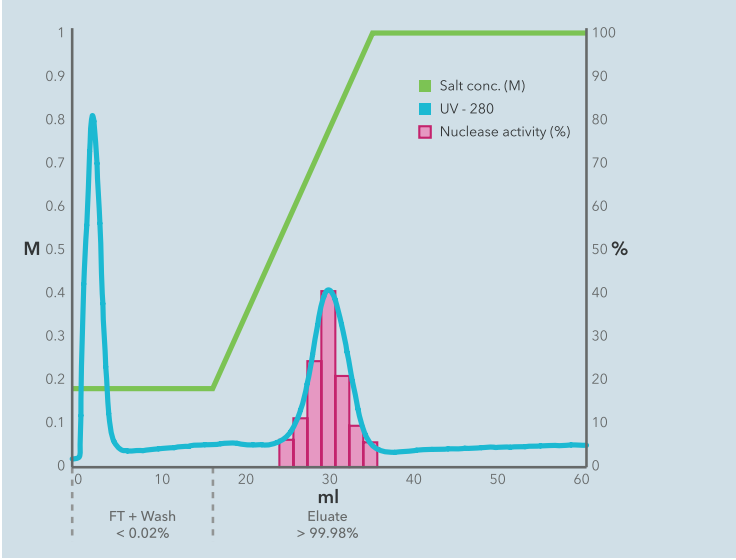

Fig. 3: Salt Active Nuclease (SAN) binds tightly to SP-sepharose columns at pH 9.0 with 0.2 M salt (less than 0.02 % leakage).

Salt Active Nuclease High Quality Bioprocessing Grade

- For efficient removal of nucleic acids during biomanufacturing of proteins, viral vaccines and gene therapy viruses
- Simplifies workflow and reduces costs
- Quality dossier and TSE/BSE, Allergen, Aflatoxin and GMO certificates available
- SAN HQ ELISA for demonstrating removal of SAN HQ
- For further ordering information please inquire

Ordering Information

Product	Size	Cat. no.
Salt Active Nuclease	5000 U	18541.01

*Solution in 25 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 0.5 M NaCl, 0.01 % Triton® X-100, 50 % (v/v) glycerol)

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