SAFC®

Pharma & Biopharma Raw Material Solutions

Data Sheet

Benzonase® Salt Tolerant endonuclease Emprove® Expert

The best-in-class solution for nucleic acid removal at high salt concentrations.

Key features:

- A new endonuclease designed for viral therapy manufacturing
- Active at higher salt concentration, up to 1M NaCl
- Non-animal origin, recombinant from *E.Coli*, High Purity
- GMP (IPEC-PQG) and Emprove® Expert, FDA DMF
- Devoid of glycosylations and post-translational modifications
- Precise detection via proprietary immunoassay
- Custom options on demand (CoA, Packaging)
- Compatible with Deviron® detergents for cell lysis

MilliporeSigma

Enzyme Characteristics

Benzonase® Salt Tolerant endonuclease Emprove® Expert is a newly developed enzyme, that differs in the amino acid sequence from other Benzonase® endonucleases. It was designed with state-of-the-art protein engineering capabilities to ensure the highest activity at high salt concentrations.

The protein is a monomer with a molecular weight of about 27 kDa and a an isoelectric point (pI) at pH 9.68. It is functional between pH 6 and 10 and from 0 °C to 37 °C. Mg2+ (1–2 mM) is required for enzyme activity as with other Benzonase® endonucleases.

When standard endonucleases are losing their activity beyond 200 mM salt, the Benzonase® Salt Tolerant endonuclease Emprove® Expert shows superior performances (Figure 1).

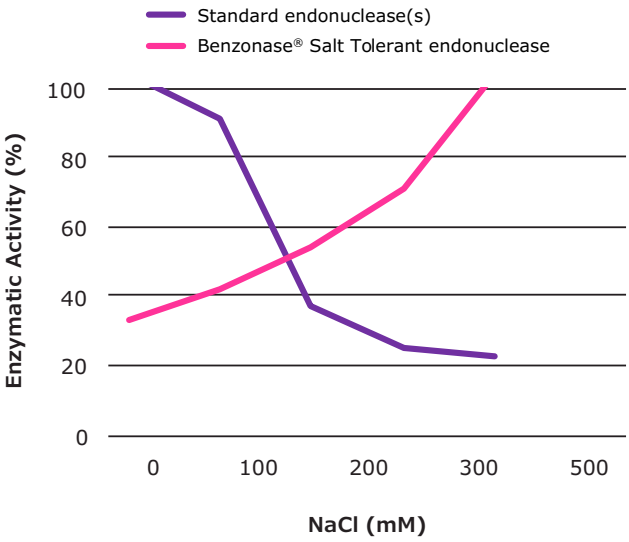


Figure 1:
Enzymatic activity (%) in the presence of NaCl (mM).

Substrate Specificity

Benzonase® Salt Tolerant endonuclease Emprove® Expert degrades both DNA and RNA, whether single stranded, double-stranded, linear, circular or supercoiled. No base preference is observed.

As with all endonucleases, **Benzonase® Salt Tolerant endonuclease Emprove® Expert** hydrolyzes internal phosphodiester bonds present between the nucleotides. Upon complete digestion, all free nucleic acids present in solution are reduced to 5'-monophosphate terminated oligonucleotides, which are three to five bases in length.

Benzonase® Salt Tolerant endonuclease digests DNA to undetectable levels, below 10 base pairs (Figure 2).

To ensure full compliance with regulatory requirements for nucleic acid clearance in drug products, the digestion, the digestion potential of **Benzonase® Salt Tolerant endonuclease** has been tested with different NaCl concentrations.

FDA guidance* regarding DNA contaminants in human gene therapy manufacturing can be summarized as such:

- Any residual DNA associated with viral vectors needs to be minimized.
- Residual DNA for continuous non-tumorigenic cells should be limited to less than 10 ng/dose and DNA fragments size should be below 200 base pairs.

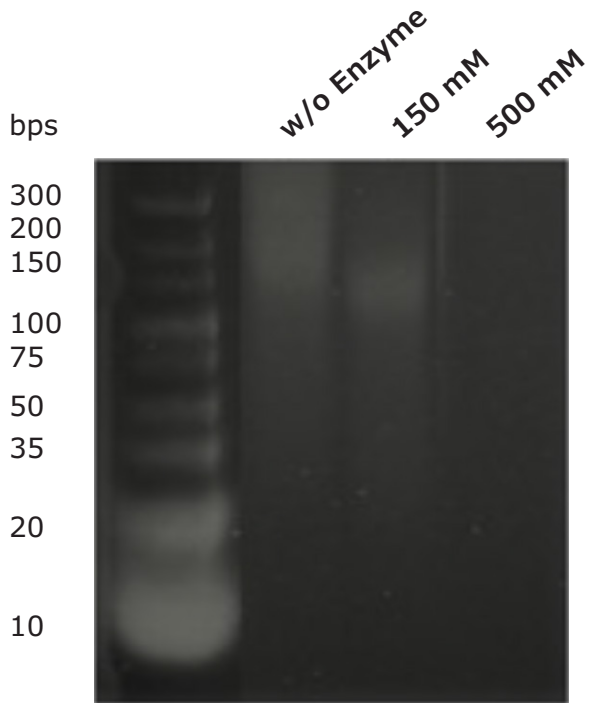


Figure 2:
Benzonase® Salt Tolerant endonuclease DNA digestion efficiency at 150 and 500 mM NaCl concentrations – 4% agarose gel electrophoresis.

Source* Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) – Guidance for Industry, JANUARY 2020
https://www.fda.gov/media/113760/download

Protease activity

All Benzonase® endonuclease references are **free of detectable protease activity**, making these enzymes ideal for production processes in which high yields of biologically active proteins are desired. The absence of proteolytic activity is monitored by a highly sensitive and validated assay using a resorufin-labeled casein.

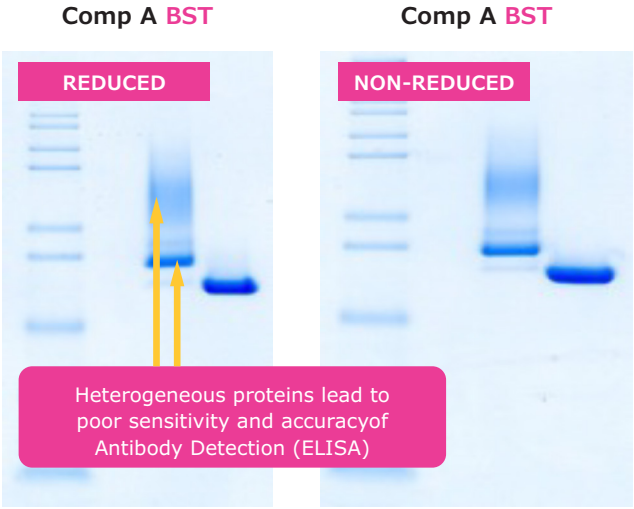
Product purity

Like Benzonase® endonuclease Emprove® Expert (1.10695/1.01697) and Benzonase® endonuclease Safety Plus Emprove® Expert (1.03773), **Benzonase® Salt Tolerant endonuclease Emprove® Expert** >99% purity is achieved by chromatographic purification.

The preparation does not contain any antimicrobial preservatives or protein stabilizers except glycerol (of synthetic origin). The solution is filtered through a 0.2 µm PVDF (Polyvinylidene fluoride) filter before final fill.

No post-translational modifications

Benzonase® Salt Tolerant endonuclease Emprove® Expert is produced in a bacterial production platform, therefore the final product is devoid of post-translational modifications, ensuring uniform size and activity between batches and reliable detection via monoclonal antibodies (ELISA assay).



BST: Benzonase® Salt Tolerant endonuclease **Competitor/ Comp A**: Benchmark endonuclease active at high salt.

Figure 3:
Identity and purity of Benzonase® Salt Tolerant endonuclease and competitor A as shown by non-reducing and reducing SDS-PAGE.

Key protein features

- **Production in *E.Coli***
- High Purity
- No post-translational modifications
- High batch to batch reproducibility

Benzonase® Salt Tolerant endonuclease has a precisely defined molecular size (Figure 3).

Operating Conditions

Benzonase® Salt Tolerant endonuclease Emprove® Expert retains its activity under a wide range of operating conditions, as specified in Table 2.

NB: No drastic change in operating ranges compared to legacy Benzonase® endonuclease references is observed outside of the monovalent cation concentration parameter.

Condition	Optimal*	Effective†
Mg2+	1-2 mM	1-10 mM
pH	8.0-9.2	6-10
Temperature	37°C	0-37°C
Monovalent cation concentration	500-1000 mM	200-1000 mM

Table 2: Benzonase® Salt Tolerant endonuclease operating conditions.

* ”Optimal” is defined as the condition under which Benzonase® endonuclease retains > 90% of its activity.

† ”Effective” is defined as the condition under which Benzonase® endonuclease retains > 15% of its activity.

Benzonase® Salt Tolerant endonuclease Emprove® Expert activity is not affected by the presence of ionic and non-ionic detergents. It is compatible with DEVIRON® detergents for cell lysis applications.

To ensure the complete digestion of residual nucleic acid under different detergent conditions, agarose gel electrophoresis was ran in parallel. **Benzonase® Salt Tolerant endonuclease** efficiently digest DNA at 500 mM NaCL concentration with all tested detergents.

Detergent	Detergent Concentration	Impact on enzymatic activity
Deviron® 13-S9	1%	Null
Deviron® C16	1%	Null
Polysorbate 20	1%	Null
TRITON™ X-100	1%	Null

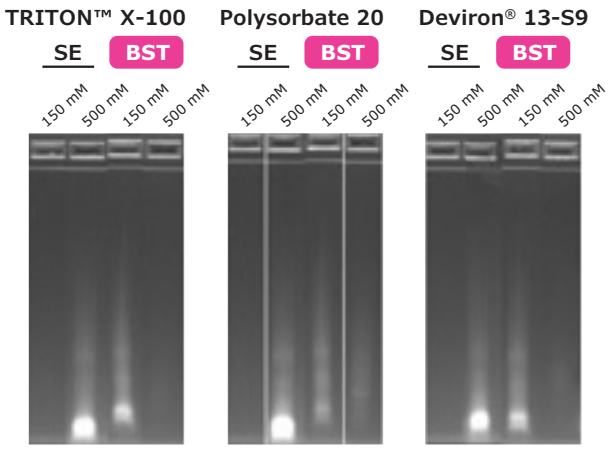


Table 3 and Figure 4: Benzonase® Salt Tolerant endonuclease enzymatic activity is not negatively affected by four detergents commonly used for cell lysis.

BST: Benzonase® Salt Tolerant endonuclease Emprove® Expert

SE: Standard Benzonase® endonuclease

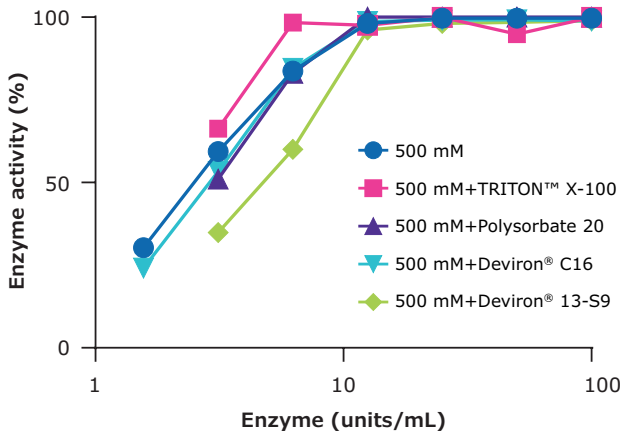


Figure 5: Benzonase® Salt Tolerant endonuclease & Standard endonuclease digestion efficiency at 150 and 500 mM NaCl concentration with 3 different detergents.

General* Procedure for HEK Cell Lysis & DNA Digestion with Benzonase® Salt Tolerant endonuclease.

Suspension or adherent HEK293 cells are cultivated accordingly and transfected with the selected plasmid polyethylenimine (PEI) complex.

After 72 hours of cultivation, cells are harvested and lysed using the following procedure:

- Detergent concentration: 0.5 % wt/vol.
- Detergents: Tween™ 20 and TRITON™ X-100, Deviron® C16 and Deviron® 13-S9
- **Nuclease: 25 U/mL Benzonase® Salt Tolerant endonuclease with 2 mM $MgCl_2$**
- Lysis time: 2 h
- Temperature: 37 °C
- 5 % CO_2

*Because results are process specific, we strongly recommend process specific trials to determine optimized process conditions. Our experts can assist you with this step.

For more application data, check our poster on AAV process intensification using high salt lysis & Benzonase® Salt Tolerant endonuclease.

Benzonase® Salt Tolerant endonuclease Frequently Asked Questions.

1. Is the amino acid sequence similar to legacy Benzonase® products like the Benzonase® Safety Plus?

The Benzonase® Salt Tolerant endonuclease is a new enzyme designed using state-of-the-art protein engineering to ensure the highest activity at high salt concentration. It contains a different amino acid sequence compared to other Benzonase® legacy products.

2. What are the differences regarding operating conditions with legacy Benzonase® products?

As an endonuclease, the Benzonase® Salt Tolerant endonuclease operates with the same conditions as legacy Benzonase® products. The exception is for monovalent cation concentration, where we recommend a minimum of 200 mM NaCl.

3. How do I inhibit this enzyme?

The enzyme can be inhibited by addition of EDTA or exposure to heat (>52°C for 10 minutes).

4. How do I remove this enzyme?

Procedures used to remove the legacy Benzonase® enzyme will also remove the Benzonase® Salt Tolerant endonuclease. Tangential Flow Filtration is the most common one.

5. How do I detect traces of this enzymes?

Like for our legacy Benzonase® endonucleases, a dedicated ELISA kit can be used to ensure a robust detection of Benzonase® Salt Tolerant endonuclease.

6. Why should I use this enzyme instead of other standard nucleases?

Commercially available standard nucleases used for bioprocessing have a similar amino acid sequence. It means their ability to digest nucleic acids at high salt is similar and considered not optimal.

Their effective operating conditions for monovalent cation concentrations range between 0 and 200 mM which is comparable to the physiological salt concentrations found in biological processes.

Use of salt concentrations above physiological levels during HEK cell lysis has a significant impact on the overall process yield In our studies, cell lysis at 500 mM NaCl results in infectivity and overall AAV yields that are significantly higher compared lysis at 150 mM salt concentration.

Therefore, use of a salt tolerant nuclease is essential to remove nucleic acid in these high salt conditions and maintain the safety of the final drug product.

7. Can I still use regular nucleases at salt concentrations >200 mM?

Activity of regular nucleases at higher than physiological salt concentrations will be drastically reduced or completely inactivated, leading to the need to add more enzymes, burdening the the purification process and increaseing costs. For that reason, we recommend the use of a salt tolerant nuclease.

8. How is high salt involved with aggregation phenomena?

Use of a high salt concentration reduces AAV aggregation and facilitates removal of sticky DNA from the AAV capsids. Ionic strength and buffer composition affect the electric double layer (EDL) surrounding viral particles, compressing, or expanding it and leading to different electrostatic phenomenon.

9. Can I use detection kits from other suppliers to detect the Benzonase® Salt Tolerant endonuclease, such as those specialized for other “salt active” nucleases?

The Benzonase® Salt Tolerant endonuclease is a new enzyme and only our proprietary detection kits can be used.

Any product not sold via an official MilliporeSigma channel, where the trade name “Benzonase®” is used, should be considered a counterfeit product.

Benzonase® Salt Tolerant endonuclease Frequently Asked Questions. *(continued)*

10. Why is the expression host so important for enzyme production?

Protein expression can be achieved using multiple production platforms with eukaryotic and procaryotic based systems being the most common. Resulting products are not similar, however, and post-translational modification profiles are different depending on the platform used.

Proteins produced using yeast expression host are heavily modified post-translationally (e.g., glycosylated) which can lead to various issues during product usage such as poor detection accuracy with antibody assays.

NB: Other salt active endonucleases on the market are expressed in yeast and heavily glycosylated.

11. Why can post-translational modification of nucleases impact the detection accuracy and efficiency?

Glycosylated proteins have a non-homogeneous profile, with high batch to batch variability. As standard detection methods use immunoassays, antibodies created to detect the protein of interest cannot cover the full spectrum of modifications that result from yeast expression. This leads to a poor detection accuracy of antibody-based detection methods such as ELISA Kits.

12. Can you help me define which nuclease would be the most cost effective for my process and deliver the desired process efficiency and yield?

We have an entire portfolio of nucleases to meet your regulatory and process condition needs. Do not hesitate to request our MSAT (Manufacturing Science and Technology) support for your process development. Our team has expertise across the full manufacturing workflow, from upstream processing to final fill and finish.

Ordering Information

Benzonase® Salt Tolerant endonuclease EMPROVE® EXPERT

Designation	Package Size	Order No.
Benzonase® Salt Tolerant endonuclease EMPROVE® EXPERT	80,000 U/vial	1.04445.1010
Benzonase® Salt Tolerant endonuclease EMPROVE® EXPERT	400,000 U/vial	1.04445.0001
Benzonase® Salt Tolerant endonuclease EMPROVE® EXPERT + Tailgate sample in same outer package	4,000,000 U/vial + 40,000 U	1.04445.0010

Legacy Benzonase® endonuclease products

Designation	Package Size	Order No.
Benzonase® endonuclease, for biotechnology	100,000 U/vial	1.01654.0001
Benzonase® endonuclease, for biotechnology	500,000 U/vial	1.01656.0001
Benzonase® endonuclease, suitable for biopharmaceutical production EMPROVE® EXPERT	100,000 U/vial	1.01695.0001
Benzonase® endonuclease EMPROVE® EXPERT	500,000 U/vial	1.01697.0001
Benzonase® endonuclease EMPROVE® EXPERT	5,000,000 U/vial	1.01697.0010
Benzonase® endonuclease Safety Plus EMPROVE® EXPERT	100,000 U	1.03773.1010
Benzonase® endonuclease Safety Plus EMPROVE® EXPERT	500,000 U	1.03773.0001
Benzonase® endonuclease Safety Plus EMPROVE® EXPERT + Tailgate sample in same outer package	5,000,000 U/vial + 50,000 U	1.03773.0010
Benzonase® endonuclease ELISA Kit III for the immunological detection of Benzonase® endonuclease	5 plates (8x12) plus reagents	1.04358*
Benzonase® endonuclease ELISA	1 plate or 5 plates (8x12) plus reagents	EZBNZ3-160K

** 1.04358.001 for all countries except US and Canada (1.04358.002)*
***Note** 1.04358 & ESBZN3-160K are not effective for the detection of Benzonase® Salt Tolerant endonuclease (1.04445). A dedicated detection kit will join the portfolio in 2024.*

Deviron® detergent portfolio

Designation	Package Size	Order No.
Deviron® 13-S9 EMPROVE® EXPERT	1 L	1086941000
Deviron® 13-S9 EMPROVE® EXPERT	2.5 L	1086942500
Deviron® 13-S9 EMPROVE® EXPERT	25 L	1086949025
Deviron® C16 EMPROVE® EXPERT	1 L	1086931000
Deviron® C16 EMPROVE® EXPERT	2.5 L	1086932500
Deviron® C16 EMPROVE® EXPERT	25 L	1086939025

For additional information, please visit
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MS_DS13431EN Ver. 1.0
03/2024