Benzonase® endonuclease

Balancing efficiency and regulatory compliance — the smart solution for DNA removal in biopharmaceutical production
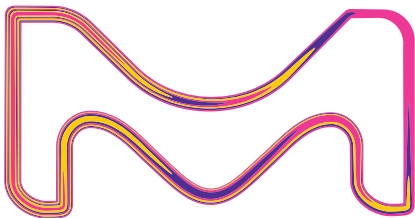
SAFC®

Pharma & Biopharma Raw Material Solutions

Table of Contents

Benzonase® endonuclease

Perfectly poised for compliance and higher yields

Meeting the strictest possible purity standards for biopharmaceuticals is a challenge on its own. Want to raise the bar even higher and increase your downstream processing yields at the same time? Fortunately, there is Benzonase® endonuclease. It's the one effective method to remove both DNA and RNA, whether you're in the lab or working with industrial-scale processes.

Our unique, genetically engineered endonuclease, Benzonase® endonuclease offers a variety of advantages over existing methods of nucleic acid removal. You will see it at work in various applications throughout the pharmaceutical and biotechnological industries. And Benzonase® endonuclease has proven its value in the lab for over 30 years.

Benzonase® endonuclease also offers you the reliability and high quality you have come to expect from us — because it's manufactured under GMP conditions and exclusively distributed by Merck and our associates. We invite you to discover how Benzonase® endonuclease can help you work more efficiently.

About Benzonase® endonuclease

What are the benefits of Benzonase endonuclease?

- The capability to attack and degrade all forms of DNA and RNA
- The absence of detectable proteolytic activity
- A wide range of operating conditions
- The advantages of exceptionally high specific activity
- The assurance that you will meet industry requirements for reliable supply and consistent high quality thanks to IPEC-PQG GMP manufacturing
- Family of Benzonase® products offering the suitable purity and documentation for use from research up to highly regulated biopharmaceutical manufacturing

- Supported by Emprove® Dossiers
- The support by our FDA Bulk Biological Master File (FDA Reg. No. BBMF 5403; available in eCTD format) for 101695 & 101697 to be cited in your regulatory filing
- The availability in bulk quantities for the use in R&D up to manufacturing scale
- A guideline on its use and its removal from biopharmaceutical processes

Benzonase® endonuclease has been specially designed for applications in biotechnological processing, such as:

- Purification of viral vaccines, viral vectors for vaccine, cell and gene therapy, and oncolytic viruses
- Removing DNA/RNA from proteins and other biologicals
- Reducing viscosity caused by nucleic acids

- Preparing samples in electrophoresis and chromatography
- Preventing cell clumping

Why Benzonase® endonuclease is the right choice

When you choose Benzonase® endonuclease, you are choosing an endonuclease that ensures exceptionally high purity and activity for your finished product. This enzyme has been designed to be supplied without protease activity and without the viral contaminants that can accompany enzymes isolated from natural sources. To meet the widest possible range of processing and cost requirements, Benzonase® endonuclease is available in different grades of purity:

- Benzonase® endonuclease Safety Plus Emprove® Expert
- Benzonase® endonuclease Emprove® Expert
- Benzonase® endonuclease

Benzonase® endonuclease is a genetically engineered endonuclease produced in *Escherichia coli* (*E. coli*) strain W3110, a mutant of strain K12, which contains the proprietary pNUC1 production plasmid. This plasmid encodes an endonuclease normally expressed in *Serratia marcescens.*

Which Benzonase® endonuclease to choose:

All Benzonase® products supply you with the same Benzonase® enzyme isolated from *Serratia marcescens*, proven by LC-MS-MS. Products vary in purity, non-animal origin certification, and analytical technologies applied for product release as shown in the **Table 1**.

Features / Application	Benzonase® endonuclease (purity grade II => 90%)	Benzonase® endonuclease Emprove® Expert	Benzonase® endonuclease Safety Plus Emprove® Expert
Origin	*Serratia marcescens*, Production: *E. coli* K12 strain W3110; 30 kDa; PI 6.85; sequence homology proven		
Purity	=> 90%	=> 99%	=> 99%
GMP manufacturing process according to ICHQ7*; release according to IPEC-PQG GMP	No	Yes	Yes
FDA Bulk Biological Master File (US-BBMF) & Emprove® Dossiers	No	Yes	Yes
Non-Animal-Origin (NAO), recombinant from *E. coli* in chemically defined production medium	No	No	Yes
Shipment with temperature strips	No	No	Yes
Tailgate samples for large pack size	No	No	Yes (with 5M unit size)
Lot release *in vitro* test for absence of adventitious viruses (3 cell lines) and Mycoplasma test	No	No	Yes
Endotoxins (LAL) microbial testing	Not specified < 10 CFU/100,000 U	< 0.25 EU/1,000 U < 10 CFU/100,000 U	< 0.25 EU/1,000 U < 10 CFU/100,000 U
Target customer	Research	Viral Vaccines	Viral & Gene Therapy
Long term product availability	All Benzonase® products will stay in portfolio		

Table 1. Different Benzonase® endonuclease products

Meets the highest standard

U.S. FDA regulations are regarded as one of the world's strictest standards for biopharmaceuticals. To date, the U.S. FDA has not issued any explicit regulations governing the production and use of process materials such as Benzonase® endonuclease. Although they do not appear on the final dosage form, these process materials are used in the direct purification of active pharmaceutical ingredients.

In other words, finished biopharmaceutical products that are safe call for process materials that are safe, too. At MilliporeSigma, we never compromise on safety. That's why Benzonase® endonuclease is manufactured under GMP conditions. The U.S. FDA also stocks a DMF type II file for Benzonase® endonuclease item 101695 and 101697 (Reg. No. BBMF 5403; current version 2013), available in eCTD format.

A complete package

The finished product does not contain additives of animal origin, such as stabilizers like bovine serum, albumin or gelatin. Benzonase® endonuclease is supplied in 50% glycerol solution to prevent the preparation from freezing. The glycerol is of synthetic origin. During production of Benzonase® endonuclease, the fermentation medium contains casamino acids from considering non-animal origin 103773. Synthetic amino acids mix replacing Casamino acids this milk is considered fit for human consumption. Please don't hesitate to ask us for full batch documentation that meets U.S. FDA requirements.

The Emprove® Program – Your Fast Track through Regulatory Challenges

The Emprove® Program complements our product portfolio, and provides three types of dossiers to support different stages of development and manufacturing operations such as qualification, risk assessment, and process optimization. This program consolidates comprehensive product-specific testing, quality and regulatory information, making it readily available to our customers to simplify their compliance needs.

Viral safety

Depending on your application, you can choose between our regular Benzonase® endonuclease and Benzonase® endonuclease Safety Plus Emprove® Expert. Both are prepared in a well defined bacterial expression system (*E. coli* strain W3110) leading to a minimal risk of viral contamination. Additionally, Benzonase® endonuclease Safety Plus Emprove® Expert is produced in a completely chemically defined fermentation medium without any hydrolysates and every batch is tested for the absence of relevant adventitious viruses and mycoplasma.

* Manufacturer's declaration

Microbiological safety

Each batch of Benzonase® endonuclease, purity grade I and II containing 100,000 or more enzyme units, is tested using a modified EP method to ensure it only contains trace levels (< 10 CFU in 100,000 U) of aerobic bacteria, yeasts and molds.

Endotoxin testing

Each batch of Benzonase® endonuclease, purity grade I containing 100,000 or more enzyme units, is tested for endotoxins using the well-known LAL test. The total endotoxin level is below 0.25 EU per 1,000 units.

Product quality assurance:

Using Benzonase® endonuclease to remove DNA/RNA

U.S. FDA guideline[1] for the manufacture of recombinant biologicals for therapeutic use demand that nucleic acid contamination should be limited to 100 pg per dose (in the end product). Benzonase® endonuclease, when used under appropriate reaction conditions, will degrade all nucleic acid sequences down to oligonucleotides of approximately three to five base pairs in length—which is significantly below the hybridization limit—enabling recombinant proteins to meet the FDA guidelines for nucleic acid contamination.

[1]Guidance for Industry, Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications, U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research [February 2010]

Cell disintegration:

Using Benzonase® endonuclease to reduce viscosity

The ability of Benzonase® endonuclease to rapidly hydrolyze nucleic acids makes the enzyme an ideal choice for reducing cell lysate viscosity—both in the research laboratory and the manufacturing plant.

Using Benzonase® endonuclease to reduce viscosity also allows you to:

- Reduce processing time
- Increase the yield of protein products
- Improve the separation of pellets and supernatants in centrifugations
- Facilitate the filtration of solutions, especially ultrafiltration
- Increase the efficiency of chromatographic purification steps (e.g., in expanded bed adsorption)
- Boost the productivity of cross-flow microfiltration steps (e.g., in inclusion body processing)

Benzonase® endonuclease may be used with all methods of cell lysis, including lysozyme treatment, freeze-thawing procedures, and high-pressure homogenization. Although Benzonase® endonuclease may be added post-lysis, it was shown that, when the same amount of Benzonase® endonuclease is added before lysis instead of afterwards:

- The amount of Benzonase® endonuclease required for nucleic acid hydrolysis can be reduced 50 to 200 times
- Viscosity reduction will occur significantly faster

Particle processing:

Using Benzonase® endonuclease to facilitate particle purification

It is well known that nucleic acids may adhere to cell-derived particles, such as viruses or inclusion bodies. This adhesion may interfere with separation due to agglomeration, change in particle size or change in particle charge, resulting in a reduced product yield. Benzonase® endonuclease is well suited for reducing the nucleic acid load during purification, thus eliminating interferences and improving both yield and purity of the end product.

Bioanalytical applications:

Using Benzonase® endonuclease for sample preparation

Bioanalytical applications of Benzonase® endonuclease include sample preparation for ELISA, chromatography or two-dimensional electrophoresis (protein mapping), and footprint analysis. Benefits of sample treatment include improved resolution and increased recovery of samples.

Enzyme Characteristics

Benzonase® endonuclease is designed to help your products meet the world's most exacting standards for biopharmaceuticals. So it's to your advantage to know exactly what makes Benzonase® endonuclease work the way it does.

General profile

All Benzonase® endonuclease products contain the enzyme of the same amino acids sequence differing in purity, non-animal origin level and analytical package. The protein consists of two subunits with a molecular weight of about 30 kD each. The protein has an isoelectric point (pI) at pH 6.85. It is functional between pH 6 and 10, and from 0 °C to above 42 °C. Mg^{2+} (1–2 mM) is required for enzyme activity.

Substrate specificity

Benzonase® endonuclease acts as an endonuclease that degrades both DNA and RNA—whether single-stranded, double-stranded, linear, circular or supercoiled. No base preference is observed. As with all endonucleases, Benzonase® endonuclease 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.

Product purity

Analysis of Benzonase® endonuclease, purity grade II (=> 90%) by SDS-PAGE results in a dominant band corresponding to Benzonase® endonuclease. All other proteins present (< 10%) are derived entirely from *E. coli*. Benzonase® endonuclease Emprove® Expert and Benzonase® endonuclease Safety Plus Emprove® Expert is produced by chromatographic purification of Benzonase® endonuclease, purity grade II. Analysis of Benzonase® endonuclease, purity grade I by SDS-PAGE results in a single band corresponding to Benzonase® endonuclease. All other proteins present (< 1%) are derived entirely from *E. coli*. The preparation does not contain any antimicrobial preservatives or protein stabilizers except glycerol (of synthetic origin). The solution has been filtered through a 0.2 μm filter.

Protease activity

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

Operating conditions

Benzonase® endonuclease retains its activity under a wide range of operating conditions, as specified in **Table 2** and **Figures 1 and 2**. As illustrated in **Figure 3 to 6** Benzonase® endonuclease is active over a wide pH range and in the presence of different inorganic salts. **Figures 7 to 10** demonstrate the stability in the presence of ionic and non-ionic detergents, urea and ammonium sulfate.

Benzonase® endonuclease reaction conditions

Condition	Optimal*	Effective†
Mg^{2+}	1–2 mM	1–10 mM
pH	8.0–9.2	6.0–10.0
Temperature	37 °C	0–42 °C
Dithiothreitol (DTT)	0–100 mM	> 100 mM
2-Mercaptoethanol	0–100 mM	> 100 mM
Monovalent cation concentration (Na^+, K^+, etc.)	0–20 mM	0–150 mM
PO_4^{3-} concentration	0–10 mM	0–100 mM

Table 2.

* "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® endonuclease activity

Specific activity for Benzonase® endonuclease is measured under standard assay conditions.

- Minimum specific activity for Benzonase® endonuclease, is 1,000,000 units/mg protein.
- Minimum specific activity for Benzonase® endonuclease Emprove® Expert and Benzonase® endonuclease Safety Plus Emprove® Expert, is 1,100,000 units/mg protein

Unit definition

A standard assay was developed to define the activity of Benzonase® endonuclease. The procedure is based on the measurement of changes in optical density that occur when oligonucleotides are released into solution during digestion of DNA with Benzonase® endonuclease. The assay is performed using excess substrate concentration, but all other conditions are set optimum. The rate of DNA degradation is measured by precipitation of undigested DNA using perchloric acid. Based on this assay, one unit of Benzonase® endonuclease is defined as the amount of enzyme that causes a change in absorbance at 260 nm of 1.0 absorption units within 30 minutes. One unit of Benzonase® endonuclease also corresponds approximately to the amount of enzyme required to completely digest 37 µg of DNA in 30 minutes under standard assay conditions.
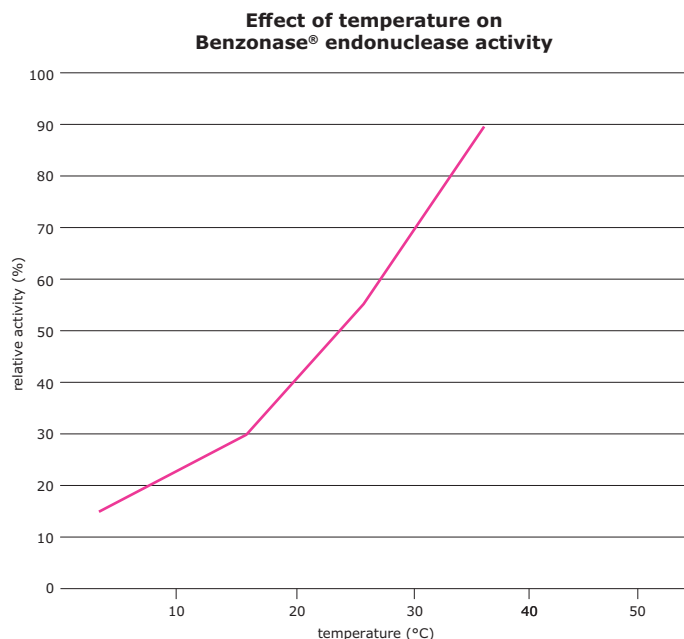
Effect of temperature on Benzonase® endonuclease activity

Figure 1.

The relative activity of Benzonase® endonuclease is rising with elevated temperature. The optimum temperature is at 37 °C.

For a detailed description of the activity assay, see Appendix.

Effect of various storage temperatures on Benzonase® endonuclease activity over time

- ●— percentage activity at -80°C (U/µl)
- ●— percentage activity at -20°C (U/µl)
- ●— percentage activity at 4°C (U/µl)
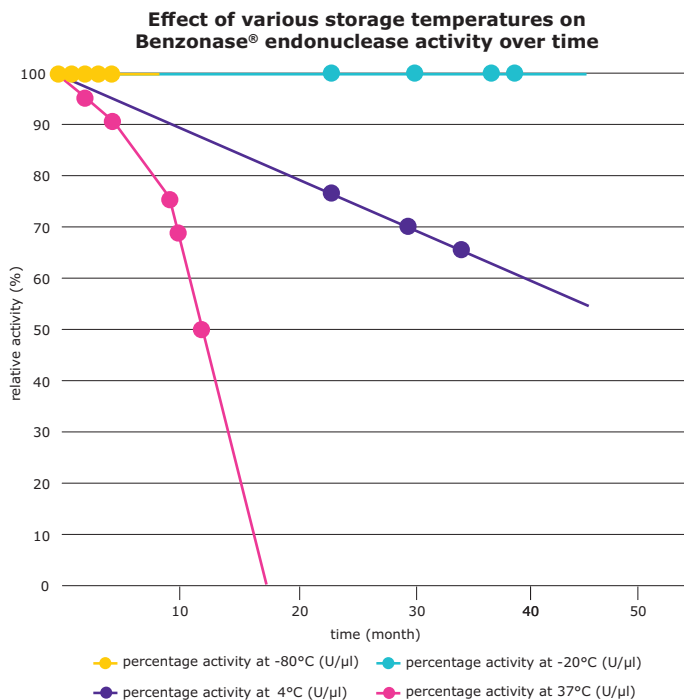- ●— percentage activity at 37°C (U/µl)

Figure 2a.

The recommended storage temperature of Benzonase® endonuclease is at -20 °C preventing loss of activity for 36 month. Storage at elevated temperatures is leading to a decrease of activity over time.

Stability of Benzonase® endonuclease stored at 25 °C/60% RH

- ●— Lot K38538697
- ●— Spec > 250 U/µl

Figure 2b.

Benzonase® endonuclease was stored in simulated primary package material at 25 °C/60% RH. The activity of lot K38538697 was measured once per week according to the monograph. After a storage time of 11 weeks the activity remained unchanged.

Temperature stability of Benzonase® endonuclease

The optimum temperature for the degradation of nucleic acids by Benzonase® endonuclease is 37 °C. The enzyme is, however, effective over a temperature range of 0–42 °C (see **Figure 1**). The optimum storage temperature is -20 °C to prevent loss of activity or freezing. The effect of storage at various temperatures is illustrated in **Figure 2a**. **Figure 2b** shows the Benzonase® endonuclease stability at 25 °C (60% RH). **Figure 2c** is showing a further proof point for the outstanding stability of Benzonase® endonuclease. No loss in activity was observed upon four freeze/thaw cycles. Although the data support stability upon this treatment we do not recommend repeated freeze/thaw cycles and storage at temperatures lower than -20 °C.
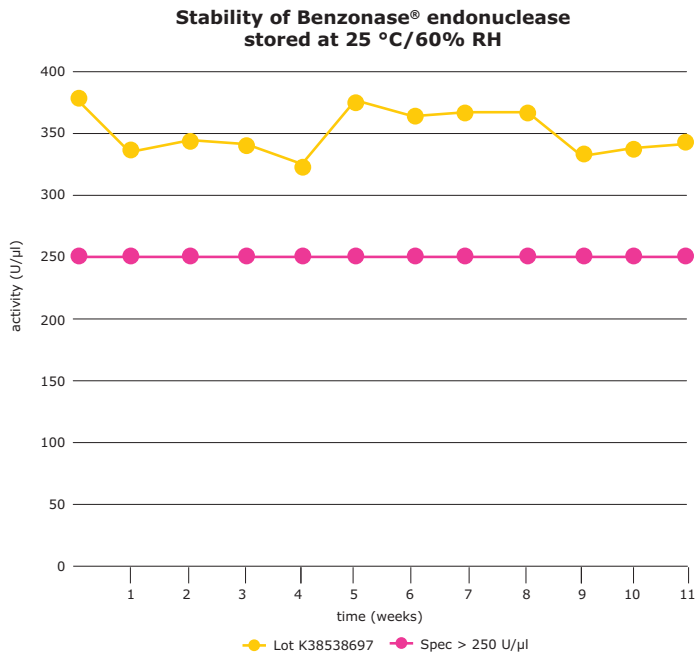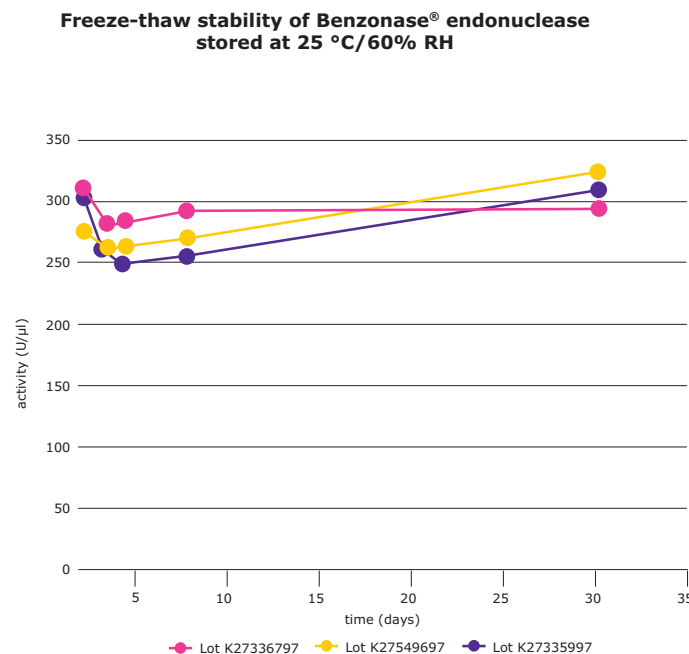
Freeze-thaw stability of Benzonase® endonuclease stored at 25 °C/60% RH

- ●— Lot K27336797
- ●— Lot K27549697
- ●— Lot K27335997

Figure 2c.

The influence of the storage temperature of -80 °C and repeated freezing and thawing on the stability of Benzonase was tested. Three batches were tested over a storage period of 4 weeks, freezing and thawing was done 4 times. The activity remained in specification, so the storage at -80 °C for 4 weeks and repeated freezing and thawing for 4 times at least is possible.
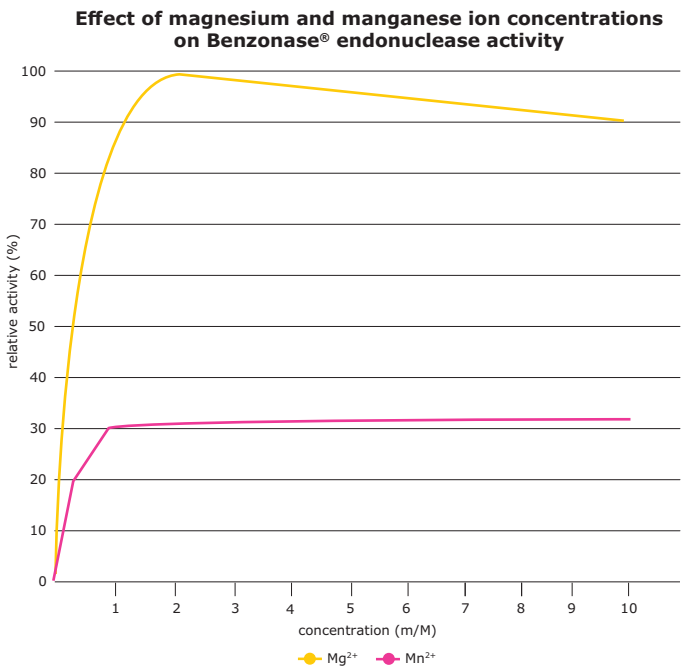
Effect of magnesium and manganese ion concentrations on Benzonase® endonuclease activity

Figure 3.

A concentration of 1 to 2 mM Mg^{2+} or Mn^{2+} is essential for activity of Benzonase® endonuclease. Mg^{2+} is preferred because it enables the enzyme to reach its optimal level of activity. Ca^{2+} and Sr^{2+} do not have effects on the activity of the enzyme.

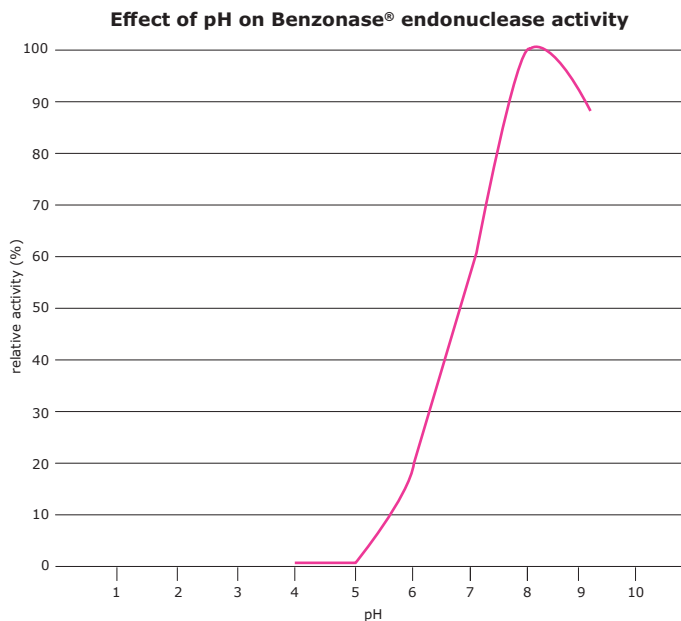**Effect of pH on Benzonase® endonuclease activity**

Figure 4.

The incubation buffers are 20 mM Tris, 20 mM histidine and 20 mM MES. In Tris, being the preferred buffer system, the pH optimum of Benzonase® endonuclease is between pH 8 and 9.2. Although not shown here, a slight buffer effect has been observed, indicating minimal changes in activity due to the effects of different buffers.

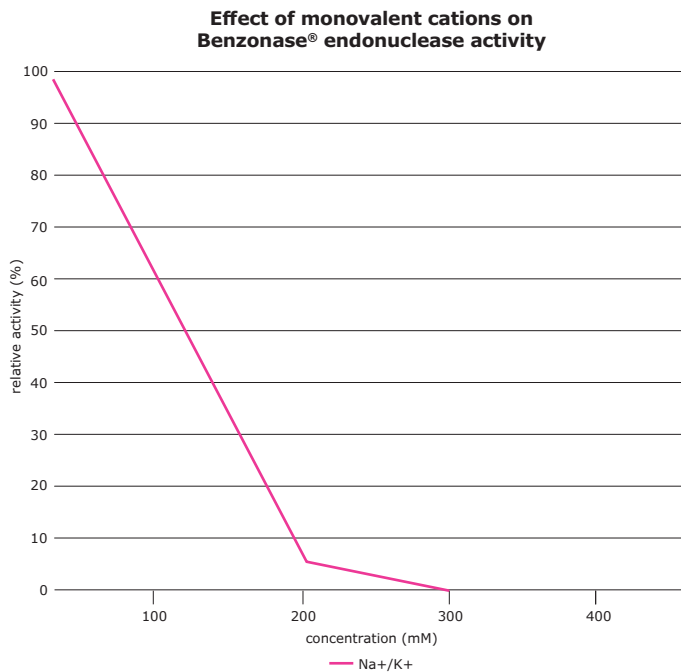**Effect of monovalent cations on Benzonase® endonuclease activity**

Figure 5.

Na^+ and K^+ inhibit the Benzonase® endonuclease activity strongly leading to a complete depletion of activity at concentrations above 300 mM. The enzyme exhibits an identical response to Na^+ and K^+, presumed that all other monovalent cations have a similar effect.

Effect of Triton® X-100

Using the standard assay for Benzonase® endonuclease activity, the effect of multiple substances frequently used in bioprocessing was evaluated. Triton® X-100 has no effect on Benzonase® endonuclease when used at concentrations below 0.4%, above an interference with the assay and the enzyme leads to inconclusive results. The presence of sodium deoxy cholate in the reaction leads to a decrease of enzyme activity with 70% of retained activity at concentrations below 0.4%.
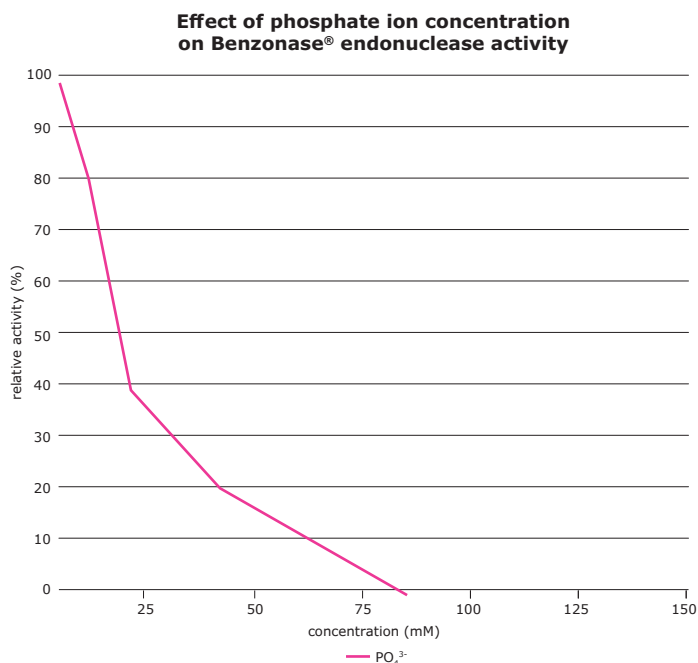
Effect of phosphate ion concentration on Benzonase® endonuclease activity

Figure 6.

The incubation buffer is a Tris-phosphate buffer. PO_4^{3-} inhibits the Benzonase® endonuclease activity strongly leading to a complete depletion of activity at concentrations above 80 mM.

Effect of detergents (Triton® X-100, sodium deoxycholate) on Benzonase® endonuclease activity
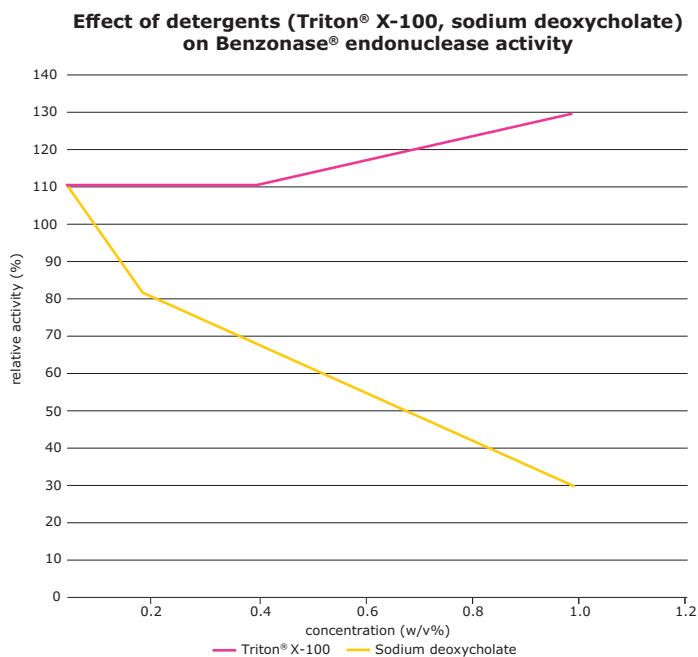

Figure 7.

The effect of detergents on Benzonase® endonuclease activity was evaluated by adding the test-substances to the reaction buffer of the standard activity assay. Concentrations of Triton® X-100 < 0.4% have no effect on the activity of Benzonase® endonuclease. At concentrations of sodium deoxycholate < 0.4%, Benzonase® endonuclease retains at least 70% of its activity.

Effect of sodium dodecyl sulfate (SDS), urea, guanidine HCl, EDTA and PMSF on Benzonase® endonuclease activity

The addition of SDS remains uncritical up to concentrations of 0.05% having no effect on the enzyme activity. Elevated concentrations of 0.1 or 1% show a stronger impact on activity due to a SDS mediated denaturation of the enzyme. Even though this effect can be partially compensated by the addition of higher Benzonase® endonuclease concentrations the activity is lost after 15 minutes of incubation at SDS concentrations of 0.1–1%.

Testing of urea revealed that the addition of concentrations up to 5 M increases Benzonase® endonuclease activity over a time span of 60 minutes with the highest activity in the first 15 minutes of the DNA decay. For concentrations of 6 M or 7 M respectively the activity drops below the urea free value after an initial activity increase during the activity measurement. The negative effect of a 7 M urea addition can be mitigated by elevated Benzonase® endonuclease concentrations but cannot prevent the loss of activity over time.

Ammonium sulfate inhibits the Benzonase® endonuclease activity strongly with 25 mM leading to an over 60% activity loss. Concentrations above 100 mM fully inhibit enzyme activity.

Moreover it was evaluated that concentrations of guanidine HCl exceeding 100 mM completely inhibit the enzyme activity. An EDTA concentration of 1 mM partially inhibits Benzonase® endonuclease. However, a concentration of 5 mM EDTA causes a more than 90% loss of enzyme activity by complexing the essential Mg^{2+} ions. PMSF in a concentration of 1 mM does not inhibit Benzonase® endonuclease
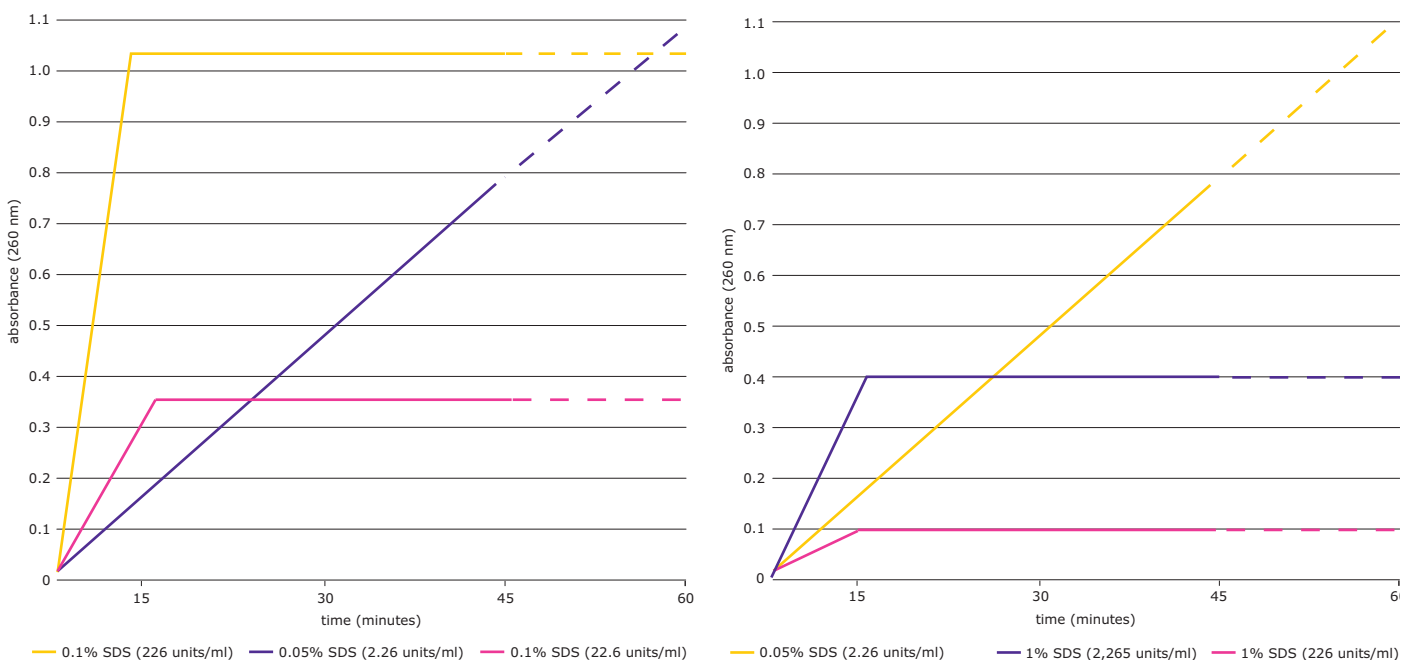
Figures 8a and 8b.

Benzonase® endonuclease retains 100% of its activity in SDS concentrations up to 0.05%. At SDS concentrations between 0.1% and 1%, Benzonase® endonuclease remains active for a short period of time before being denatured; this is illustrated by the horizontal portions of the graphs. This can be partially compensated by increasing the concentration of Benzonase® endonuclease.
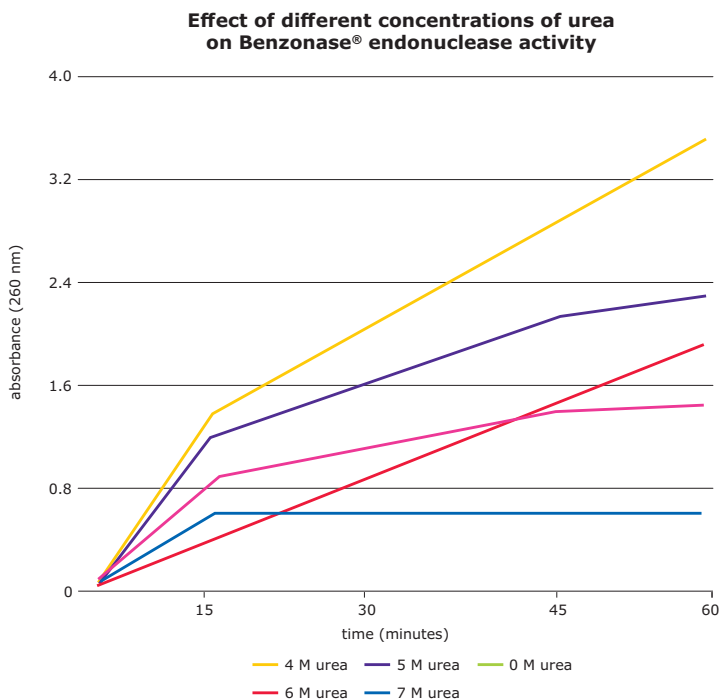
Effect of different concentrations of urea on Benzonase® endonuclease activity

Figure 9a.

Benzonase® endonuclease is activated by urea at concentrations up to approximately 5 M. Upon urea addition the enzyme activity first increases, then decreases over time. At 7 M urea, Benzonase® endonuclease denatures after 15 minutes, and activity is lost. However, significant degradation of nucleic acids occurs before the enzyme is inactivated.

Compensatory effect of different Benzonase® endonuclease concentrations at constant urea concentration
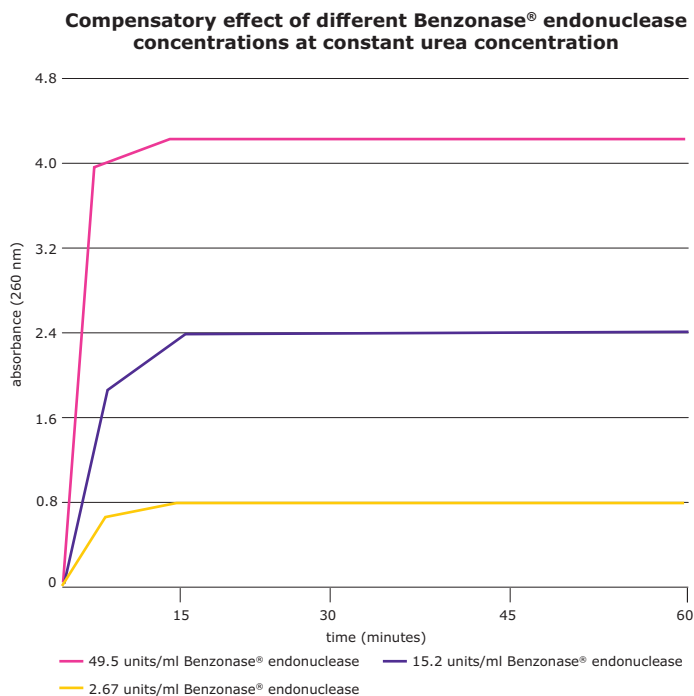

Figure 9b.

Higher concentrations of Benzonase® endonuclease can partially compensate the effects of 7 M urea but not prevent the enzyme's denaturation.

Effect of ammonium sulfate on Benzonase® endonuclease activity
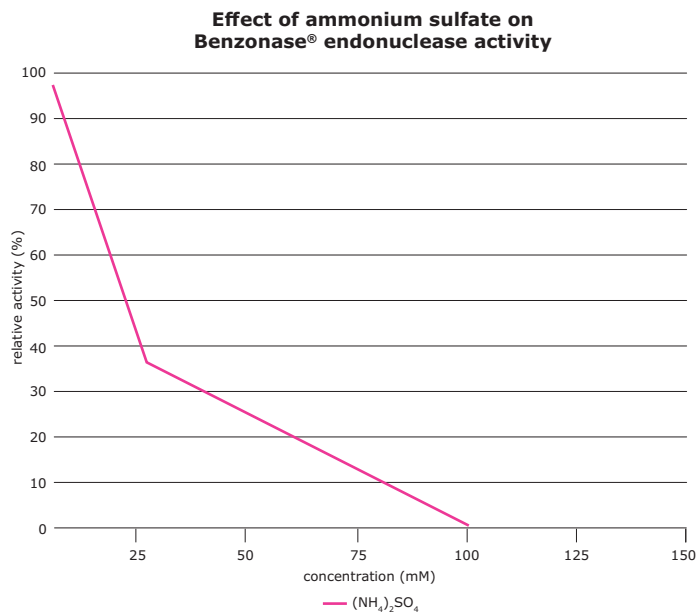
Figure 10.

Benzonase® endonuclease is inhibited by higher concentrations of ammonium sulfate, but remains active at concentrations < 100 mM.

Typical Applications

One of the great things about Benzonase® endonuclease is the many different kinds of applications you can use it in. Over the pages that follow, you will find a guide to some examples.

Although these examples do not constitute guidance on the use of Benzonase® endonuclease, they provide general information on how to address specific challenges involving residual nucleic acids in biopharmaceutical processes.

Why choose Benzonase® endonuclease?

- Possesses a higher specific activity

- Is free of detectable proteolytic activities

- Manufactured according to ICHQ7* GMP conditions in bulk quantities for large scale use released according to GMP IPEC-PQG

- Offers you the choice between our standard Benzonase® endonuclease Emprove® Expert and the non-animal origin certified and virus tested Benzonase® endonuclease Safety Plus Emprove® Expert

- Offers superior documentation including Emprove® Expert dossiers and US-BBMF file (FDA Reg. No. BBMF 5403) Please note that we have US BBMF for 101697, 101695. We do not yet have US BBMF for safety plus 103773

- Specially designed for application in biotechnological processing and biopharmaceutical production

- Used effectively in various fields of application

- Proven track record in numerous commercial vaccine manufacturing processes

Typical Benzonase® endonuclease applications

- Viral vaccine production

- Virus like particle production

- Viral vector production for vaccine and cell and gene therapy applications

- Prevention of cell clumping

- Viscosity reduction

- FAB purification

- Protein and inclusion body purification

* Manufacturer's declaration

Example 1: Elimination of nucleic acids from recombinant proteins

Operating conditions

Recombinant biopharmaceuticals must meet strict requirements concerning residual nucleic acids. This is a typical field of application for Benzonase® endonuclease, ensuring compliance with growing regulatory requirements. To demonstrate the efficiency of Benzonase® endonuclease in DNA fragment elimination, an experiment was designed using a high burden of DNA (50 µg/mL). Hence it can be regarded as a worst-case scenario, since the DNA content usually found in an operational bioprocessing environment is likely to be significantly lower. Indeed, the typical concentration of DNA encountered in bioprocesses lies between 0.5 and 5.0 µg/mL. The following experiment illustrates the influence of incubation time, temperature, and enzyme concentration on enzyme activity.

Experimental design

A solution of herring sperm DNA with a final concentration of 50 µg/mL was made up using standard test buffer (50 mM Tris-HCl, 1 mM $MgCl_2$, 0.1 mg/mL BSA, pH 8.0). The progress of the reaction was assayed as follows:
At different time intervals (0–30 hours) aliquots of 10 µL (initially containing 500 ng of DNA) were applied to a nitrocellulose membrane and hybridized with a ^{32}P-labeled probe of nick-translated herring sperm DNA. Hybridization and washing steps were carried out under low stringency conditions in order to optimize the detection of repeated sequences in the herring sperm DNA. After washing, the filter was subjected to autoradiography for one to thirty hours using an amplifying screen. DNA standards from 100 ng to 10 pg allowed a semiquantitative evaluation of the residual hybridizable DNA.

Results

Reducing the enzyme concentration while keeping all other parameters constant results in a longer incubation time. A reduction of the enzyme concentration has less influence on the initial rate than on the total time required to reach the 10 pg level. After four hours at a concentration of 90 U/mL, 99.95% of the DNA can no longer be hybridized (see **Table 3**). However, reducing the initial enzyme concentration by 90%, i.e., to 9 U/mL, still yields 99% of the DNA as non-hybridizable (see **Table 3**).

Decreasing the incubation temperature results in increased incubation times (see **Table 4**).

Since PBS is commonly used in bioprocesses, the activity of Benzonase® endonuclease in PBS compared to that in Tris was determined (see **Table 5**).

The experiment shows that complete DNA fragment elimination can be achieved using Benzonase® endonuclease. Due to its high specific activity (1×10^6 units/mg protein) it is sufficient to add the enzyme in negligible concentrations (10–100 ng/mL), even under reaction conditions far from the optimum. In conclusion, it can be said that an optimization of the conditions should be based on reaction time, temperature and concentration. Note that the three factors influence each other (see page 26, Benzonase® endonuclease triangle).

Benzonase® endonuclease concentration	Residual hybridizable DNA (in pg) after incubation for			
	0 h	4 h	6 h	22 h
90 U/mL	500,000	200	20	n. d.
9 U/mL	500,000	5,000	2,000	300

Table 3.

DNA concentration: 500 ng per sample;
Temperature: 37 °C; n. d. = not detectable (< 10 pg)

Incubation temperature	Residual hybridizable DNA (in pg) after incubation for				
	0 h	4 h	6 h	22 h	30 h
37 °C	500,000	200	20	n. d.	n. d.
23 °C	500,000	500	100	10	n. d.
0 °C	500,000	1,000	500	50	10

Table 4.

DNA concentration: 500 ng per sample; Benzonase® endonuclease concentration: 90 units/mL; n. d. = not detectable (< 10 pg)

Buffer	Residual hybridizable DNA (in pg) after incubation for				
	0 h	4 h	6 h	22 h	30 h
Tris buffer	500,000	500	100	n. d.	n. d.
PBS buffer	500,000	5,000	1,000	500	300

Table 5.

DNA concentration: 500 ng per sample; Benzonase® endonuclease concentration: 90 units/mL; Temperature: 23 °C

Example 2: Viral vaccine production – a review of selected Benzonase® endonuclease application (Review)

The application of Benzonase® endonuclease for the purification of vaccines was identified in the mid-1980s. Due to its high activity and stability it was adopted in biotechnology production templates. Today it is commonly used in vaccine manufacture to digest host cell DNA[1] meeting the requirements of less than 10 ng/dose for parenteral inoculation and less than 100 µg/dose for oral vaccines.[2; 3]

One of the early processes benzonase was used in was the production of a hepatitis A virus vaccine VAQTA® in MRC-5 cells where benzonase was applied right in the harvest after virus inactivation using Triton X-100. As outlined in the publication benzonase works in the presence of 0.1% of this nonionic surfactant. The applied concentrations are 10 µg/L,[4] which in average corresponds to about 2.8 U/mL.

In the more recent development work of the HSV-2 vaccine candindate ACAM529 in a Vero cell culture based production process Benzonase® endonuclease was applied to reduce nucleic acid impurities. The authors show that the vaccine purification is gentler and more efficient if a chemical cell lysis method is used rather than a mechanical treatment. For the Dextrane Sulfate harvest method, 90 U/mL of Benzonase® endonuclease were used and incubated for 4 hours at 25 °C. After subsequent Dead end filtration a 100% recovery of infectious titer was observed.[5]

In cell culture based manufacturing of influenza vaccines benzonase is used as Milián and Kamen outline in their review paper. In the production of Optaflu®/Flucelvax® vaccines approved in EU and US, benzonase is used before virus inactivation in this MCDK cell line based process. During Preflucel® and Celvapan® vaccines manufacturing benzonase is used in clarified harvest of the Vero cell culture to digest the host cell DNA. But also in rather emerging technologies such as MCDK based live attenuated influenza vaccines or virus like particle production in PER.C6 cells benzonase is used.[6]

An example for the use of Benzonase® endnuclease in a yeast based VLP production process is given by the production of Gardasil® vaccine against HPV. Here benzonase is added directly to the cells prior to lysis.[7] Incubation of cell-slurry with 335 U benzonase per gram yeast wet cell weight at 4 °C overnight lead to a complete digestion of DNA. In this application, a 200 mM MOPS buffer at pH 7.0 was used with Mg^{2+} concentration adjusted to 2mM.[8]

Aside the well-known applications in virus and VLP vaccines production Benzonase® endonuclease could also improve the yield in anti-bacterial vaccines such as Meningitidis vaccines. van de Waterbeemd et al. describe a production process for a native outer membrane vesicle (NOMV) vaccine against Neisseria meningitidis. The adition of 1 U/mL benzonase after the concentration step of the NOMV led to a 15-fold increase in vaccine yield compared to a reference process outlined by Pajon et al. two years ealier.[9; 10]

Application of benzonase	
VAQTA® in MRC-5 cell line	After virus inactivation step
ACAM529 in Vero cell line	In cell lysate
Optaflu®/Flucelvax® in MCDK cell line	In clarified harvest
Preflucel®/Celvapan® in Vero cell line	In clarified harvest
Gardasil® in yeast	Prior to cell lysis
NOMV from Neisseria meningitidis	After concentration step

References

1. Meeting Report – WHO Study Group on Cell Substrates for Production of Biologicals, WHO Headquarters, Geneva, 11–12 June 2007*
(*Disclaimer: This report contains the collective views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization)

2. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks Proposed replacement of TRS 878, Annex 1

3. Guidance for Industry Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD) (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852 1448

4. Patent WO1994003589 A2

5. Mundle ST, Hernandez H, Hamberger J, Catalan J, Zhou C, et al. (2013) High-Purity Preparation of HSV-2 Vaccine Candidate ACAM529 Is Immunogenic and Efficacious In Vivo. PLoS ONE 8(2): e57224.

6. Milián E, Kamen AA. Current and Emerging Cell Culture Manufacturing Technologies for Influenza Vaccines. BioMed Research International. 2015;2015:504831. doi:10.1155/2015/504831.

7. Josefsberg JO, Buckland B. Vaccine Process Technology. Biotechnology and Bioengineering, Vol. 109, No. 6, June, 2012

8. Cook JC, Joyce JG, George HA, Schultz LD, Hurni WM, Jansen KU, Hepler RW, Ip C, Lowe RS, Keller PM, Lehman ED. Purification of Virus-like Particles of Recombinant Human Papillomavirus Type 11 Major Capsid Protein L1 from Saccharomyces cerevisiae. Protein Expression and Purircation 17, 477-484 1999

9. van de Waterbeemd B, Zomer G, Kaaijk P, Ruiterkamp N, Wijffels RH, et al. (2013) Improved Production Process for Native Outer Membrane Vesicle Vaccine against Neisseria meningitidis. PLoS ONE 8(5): e65157.

10. Pajon R, Fergus AM, Koeberling O, Caugant DA, Granoff DM (2011) Meningococcal Factor H Binding Proteins in Epidemic Strains from Africa: Implications for Vaccine Development. PLoS Negl Trop Dis 5(9): e1302

Example 3: Viral vector production for cell and gene therapy – a process template and application examples of Benzonase® endonuclease

The manufacturing process of viral-vectored vaccines and viral vectors for cell and gene therapy follow a common template. In this paragraph a templated process will be described taking adenovirus manufacturing as an example. Adenoviruses (AdVs) are non-enveloped, double-stranded DNA viruses that range in size from 70–90 nm. They are very efficient at transducing target cells and can be produced at high titers.

The manufacturing process for an AdV vector is straightforward and fairly templated. At full-scale typical bioreactor size reaches 100–200 L and the overall process yields are typically 60–70%. This corresponds to a 20–30 times volume reduction after the final sterile filtration.

Products recommendations:

Media exchange: Prostak® 0.22 µm module

Media prefiltration: Millistak+® and Polysep® II 1.0/0.5 µm filters followed by filter sterilization Millipore Express® SHC filters

Clarification: Two-stage depth filtration using 3.0 µm Clarigard® polypropylene cartridge filters for large particle removal, followed by a 0.5 µm CGW6 Polysep® II glass and cellulose depth filter for small particles and colloid removal. Alternatively 0.1 or 0.22 µm Pellicon® 2 V screen cassettes/Prostak open-screen TFF modules could be applied.

Size-exclusion Chromatography: Fractogel® DEAE 650 [M] resin with Loading buffer: 50 mM Tris HCl, 100 mM NaCl, 2 mM $MgCl_2$, and 2% sucrose (pH 8.0). Washing buffer: 0.2 M NaCl. Elution buffer: 0.35 M NaCl. Resulting in >92% purity and 70–80% yield. Binding capacity of Fractogel DEAE (M) resin exceeds 5×10^{12} viral particles per mL of resin. [2]

Diafiltration: 100–300 kDa TFF Pellicon 2

Standard sampling points and tests:
- Virus seed, cell banks: Identity, purity, stability
- Crude harvest: Adventitious agents
- Purified bulk: Purity, stability
- Final product: Identity, Potency, Stability, other physical parameters.

AdV vectors can be produced using genetically engineered human cell lines that complement the deleted adenoviral genes required for replication (e.g., 293-ORF6, HEK293, PER.C6 cells). In this upstream process the cell culture media need to be exchanged when switching from cell growth to virus propagation. Typical cell concentrations during AdV infection are 0.5 to 9.0×10^7 cells/mL and the AdV titer during harvest generally ranges from 10^9 to 10^{11} pfu/mL. For the purification cells are lysed either mechanically or by a chemical lysis agent such as Triton® X-100 to harvest the viruses.

For clarification, depth filtration is typically used to remove the cells or cell debris from the AdV. Typical lysate clarification reduces the turbidity from >200 to 5–10 nephelometric turbidity units (NTU).

Carryover nucleic acid from lysed cells is a key contaminant in AdV vaccine processes. Viruses propagated in cells such as HEK293 and PER.C6 pose a greater risk of nucleic acid carry-over. According to regulatory requirements, carryover host cell nucleic acid content in the final dose of attenuated viral vaccine should be below 10 ng/dose.[1] Benzonase® endonuclease is commonly used to degrade nucleic acids (RNA, DNA, etc.) to as small as 3–5 base pairs (<6 kDa). Virus harvest treated with about 0.9–1.1 U/mL of benzonase at 30–34 °C for 4–8 hours is effective in digesting host cell DNA.

After DNA/RNA clearance, the harvest is diafiltered using TFF (100–300 kDa UF devices) to remove cleaved DNA fragments and spent nuclease (benzonase). There is a 4–10 times concentration and 5–8 N diafiltration resulting in more than 99% retention of AdVs, typically.

For further purification from trace contaminants (e.g., host cell proteins, DNA, RNA) two or three chromatography steps are employed especially in large-scale production. Ion exchange and size-exclusion chromatography can be used for purification of vectors. Next, the chromatography elute is pooled and diafiltered. Typically, 10 times concentration and 8–10 N diafiltration are performed at this step. The final purified product is filter-sterilized.

During manufacturing of viral vectors, samples are collected at multiple points for quality control (QC) testing. The manufacturing process described in this paragraph can take 5–6 weeks; 4–5 weeks for upstream processing, depending on the cell concentration; and another week beyond that for downstream processing. Virus inoculation through harvest can take up to two days; lysis and purification, up to three days; and then one day for sterile filtration and the final filling operation.

BioReliance® testing services – Benzonase® endonuclease Testing

We offer a comprehensive array of BioReliance® services and assays to support every stage of cell and gene therapy development—from viral vector manufacturing to biosafety testing in accordance with regulatory guidelines. Whether your endpoint for gene transfer is targeted for vaccines or therapeutics, quality GLP and GMP testing is offered with leading turn-around times and scientific expertise. For further information visit **BioReliance.com**.

BioReliance® services assays for final lot release with relevance to Benzonase® endonuclease application are listed in the accompanying table.

Assay No.	UK Description	Equivalent US Assay
107310GMP.BUK	Detection and quantification of residual 293 DNA in biological samples (Adenovirus type 5 production)	105112GMP.BSV
107019.BUK	PCR for the detection of residual BHK cell DNA (recombinant Adeno-Associated virus production)	105009.BSV
n/a	PCR for the detection of HSV DNA in r-AAV stocks	105505.BSV[a]
016810GMP.BUK	Enzyme Immunoassay (EIA) for the Detection and Quantitation of Residual Benzonase in Test Sample	016010.BSV

[a] This assay is only available to clients that have licensed technology from AGTC where our Carlsbad will manufacture vectors.

1. Bauer SR, Pilaro AM, Weiss KD. Testing of adenoviral vector gene transfer products: FDA Expectations. In Adenoviral Vectors for Gene Therapy. Curiel DT, Douglas JT, Eds.; Academic Press: New York, 2002; pp 615-654.

Example 4: Prevention of cell clumping by using Benzonase® endonuclease

A further application of Benzonase® endonuclease is its incorporation into cell culture media to prevent cell clumping, especially when thawing frozen cell samples. Not only is Benzonase® endonuclease free of protease activity, but it poses no threat to healthy cells, making it ideal for such a role.

Recent studies demonstrate utility of Benzonase® endonuclease in developing vaccines against some of the world's most serious infectious diseases. Peripheral blood mononuclear cells (PBMCs) isolated from whole blood have important applications in vaccine research; e.g., during quantification of vaccine-induced T-cell responses. T-cell response assay methods initially require freshly isolated cells for optimal signal detection, posing a serious practical limitation for sample handling during large clinical trials. However, frozen PBMCs (particularly PBMCs prepared from stored blood) tend to clump together upon thawing, preventing further analysis. In 2001, Smith et al.[1] demonstrated that inclusion of Benzonase® endonuclease in PBMC thawing buffer prevented cell clumping, allowing implementation of PBMC cryopreservation. This method has since been widely adopted in vaccine evaluation studies.[2, 3]

1. Smith, J.G., et al. (2001). *Clin. Diagn. Lab. Immunol.* **8**, 871.
2. Huaman, M.C., et al. (2008). *J. Immunol.* **180**, 1451.
3. Bull, M., et al. (2007). *J. Immunol. Meth.* **322**, 57.

Example 5: Use of Benzonase® endonuclease in sample preparation for protein analytics – Viscosity reduction in mammalian cell extracts

When purifying proteins, the first step is to disrupt the cells or tissue sample and extract the relevant protein fraction. The purification process should be carried out without any time delay in order to minimize losses of the target protein by proteolytic attack.

However, cell extracts often show high viscosity due to the release of nucleic acids during disintegration of cells. As a result, subsequent purification will be impeded. The first separation steps should be carried out without any time delay in order to mimimize target protein loss. The addition of Benzonase® endonuclease rapidly hydrolyzes nucleic acids, resulting in viscosity reduction thus reducing processing times and increasing protein yields.

Experimental design

Two 100 μL aliquots of a human granulocyte preparation (1.0 x 10^8 cells/mL, ca. 5 mg of total protein) were removed and centrifuged. The cells were collected and resuspended in 50 μL of a 20 mM Tris-HCl pH 9.0 buffer containing 7 M urea, 100 mM DTT, and 1% Triton® X-100. 2.5 units of Benzonase® endonuclease, purity grade I (≥ 99%) were added to sample B (see **Figure 11**). This corresponds to a final concentration of 50 units/mL. All samples were incubated at 4 °C for 5 minutes and then centrifuged at 10,000 x g for 1 minute. This experiment was repeated using two 1 mL aliquots.

Results

After centrifugation, the samples without Benzonase® endonuclease (A and C) retain a high viscosity, with no clear demarcation between supernatant and pellet (see **Figure 11**). However, the samples containing Benzonase® endonuclease (B and D) show a large reduction in viscosity. A dense pellet is formed, which allows easy removal of the supernatant. The recovery of protein was significantly increased when the sample had been treated with Benzonase® endonuclease. This can be explained by the fact that certain proteins tend to be trapped in the cell debris and viscous supernatant containing nucleic acids. During upscaling, the negative impact of high viscosity becomes even more obvious.
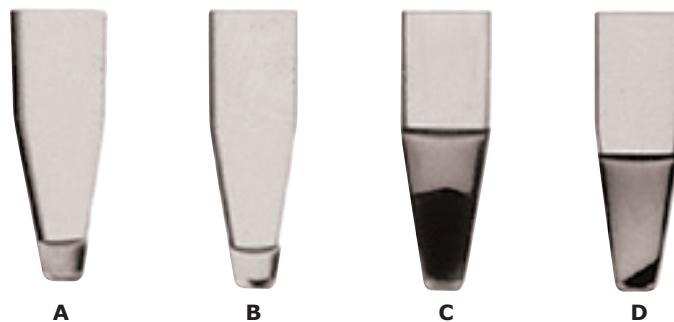

Figure 11.

Protein extracts of human granulocytes: A) without Benzonase® endonuclease, B) with Benzonase® endonuclease (5 units); C), D) same condition as in A), B), but volumes 10 times increased.

Example 6: Use of Benzonase® endonuclease in microbial expression systems – Viscosity reduction in *E. coli* cell lysates for enhanced filterability

Over the recent decades, advances in fermentation technology and cell engineering have enabled a drastic enhancement of productivity. The increase in product titer through high cell density has become routine practice for microbial expression. This strategy, however, has caused an intensification of the challenges for the primary recovery associated with high content of solids and increased level of cellular impurities in the feed stream. In particular, the presence of host cell DNA at high concentration represents a major concern for downstream purification. In this study, the addition of Benzonase® endonuclease to *E. coli* cell lysates was investigated for its potential to reduce downstream processing challenges as measured by increase in performance of a membrane filtration operation.

Experimental design

Frozen *E. coli* RV308 (*E. coli* K12 derivative) wet cell paste (wcp), re-suspended in lysis buffer (25 mM CH_3COONa, 25 mM NaH_2PO_4, 62 mM NaCl, and 5 mM $MgCl_2$ at pH 6.5, 10 mS cm^{-1}) at a ratio of wcp (g) to buffer (mL) of 0.15:1) was homogenized using a French Press at a pressure of 1,500 bar and immediately stored at 0 °C. Homogenate was clarified by batch centrifugation for 15 min at 15.000 x g generating clarified cell extract. Nucleic acid degradation by Benzonase® endonuclease grade II purity (>90%) (Cat. No. 1.01656) was analyzed at 15 °C with 200 μL samples taken at 2, 5, 10, 15, 20, and 30 min. Digestion reaction was stopped by addition of 200 μL inactivation solution (200 mM EDTA + 1.5 M Na_2HPO_4). The experiments were conducted with enzyme concentrations of 25 and 2.5 U/mL, respectively. Viscosity of *E. coli* cell lysates was measured with an Ubbelohde viscometer of type 0a which is a capillary viscosimeter with a capillary diameter of 0.53 mm. The filtration experiments were carried out in a benchtop filtration device where the cell lysate was filled into the reservoir and passed through the filter unit. Sterile syringe filters of 33 mm diameter from Merck with Polyethersulfone (PES) or Mixed Cellulose Esters (MCE) membrane were used. According to the pore size of 0.8 μm, 0.45 μm and 0.22 μm a constant pressure of 0.34 bar (5 psi), 1.4 bar (20 psi) and 2.8 bar (40 psi) was applied, respectively.

Results

High concentrations of host cell DNA lead to an increase of lysate viscosity and decreased filtration performance of cell lysates. The addition of Benzonase® endonuclease in *E. coli* clarified cell lysate led to a 50% decrease of the kinematic viscosity within 15 min of incubation time when used at a concentration of 25 U/mL. Extended incubation did not decrease the viscosity below 47% of the starting value. The 10-fold reduction in Benzonase® endonuclease concentration resulted in a lower viscosity reduction efficiency. After 30 min of incubation the kinematic viscosity only declined to 63% of the starting value. **Figure 12** shows the relative viscosity reductions achieved with the high and low concentrations of Benzonase® endonuclease as a function of incubation time.
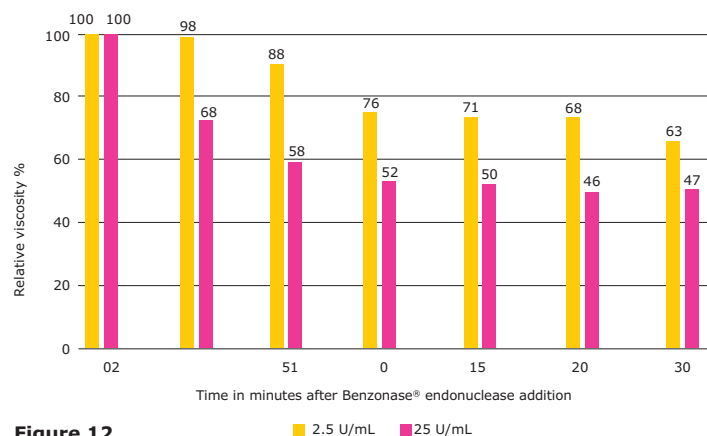

Figure 12.

Cell lysate viscosity development after addition of Benzonase® endonuclease. Change of kinematic viscosity of clarified *E. coli* cell lysate after addition of 25 U/mL or 2.5 U/mL enzyme was monitored. The 0 min sample was taken before enzyme addition. The relative viscosity was calculated by setting the values in relation to the viscosity of the untreated cell lysate (0 min sample, 100%).

The reduction in viscosity correlated with the observations made in filterability experiments. Undigested lysates (no addition of Benzonase® endonuclease) nearly immediately plugged the filters, whereas lysates treated with 25 U/mL endonuclease for 20 min at room temperature showed a drastically improved filtration performance with 0.8 μm and 0.45 μm filter units. The lower concentration of Benzonase® endonuclease gives filtration performance between the high and zero concentration conditions for the 0.8 μm membrane, as would be expected from the viscosities. After a filtration time of 20 seconds a flux of 7400 L/m2h for the 0.8 μm filter (25 U/mL Benzonase endonuclease treated) and of 4200 L/m2h for both 0.45 μm devices was reached. These findings are shown in **Figure 13**.
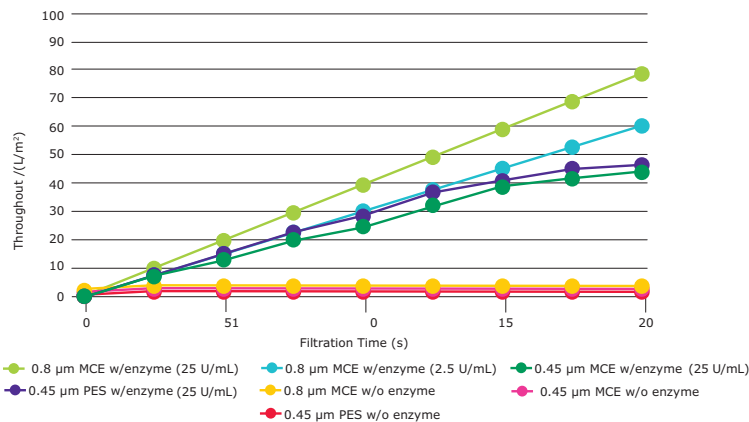
Figure 13.

Filtration of clarified *E. coli* lysate with 0.8 μm MCE as well as 0.45 μm MCE and PES filters. Lysates were treated 25 U/mL and 2.5 U/mL Benzonase® endonuclease for 20 min at room temperature respectively.

For the smaller pore sized membrane of 0.22 μm, plugging occurred immediately at the start of filtration even after digestion with 25 U/mL Benzonase® endonuclease (data not shown). To enable filtration with 0.22 μm filters, prefiltration with the 0.45 μm filter was necessary. The 0.22 μm PES filter showed the superior filtration performance after prefiltration over MCE (see **Figure 14**).
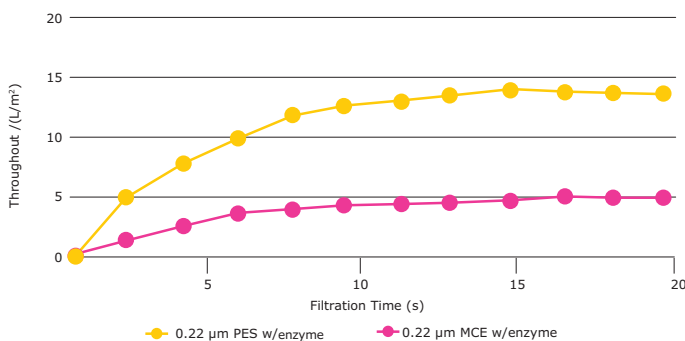

Figure 14.

Filtration of clarified and pre-filtered (0.45 μm MCE) *E. coli* lysate using 0.22 μm filters. Lysate after incubation with 25 U/mL Benzonase® endonuclease for 20 min at room temperature was used.

The faster fouling of the MCE filter could possibly be traced back to the higher binding affinity of MCE for DNA compared to PES.[1] It is also noteworthy that PES filter membranes are available from lab to process scale.

1. Liang, Z. & Keeley, A. (2013): *Filtration recovery of extracellular DNA from environmental water samples. Environmental science & technology*, 47 (16): 9324–9331.

Example 7: Use of Benzonase® endonuclease in microbial expression systems – Purification of protein fragments from inclusion bodies

Inclusion bodies can be an attractive alternative method for the production of overexpressed proteins in *E. coli*. High product yields are often possible; they are also extremely resistant to external influences and, due to their high densities, a simple but effective mechanical purification procedure is possible. However, for successful renaturation of the solubilized inclusion bodies, any adhering proteases must be completely removed. This can be impeded by the presence of large quantities of DNA in the bacterial lysate. The problem can be solved by using a combination of mechanical DNA homogenization (ultrasound) and enzymatic hydrolysis with Benzonase® endonuclease. Using this procedure, the production and purification of proteins and protein fragments otherwise sensitive to proteolytic attack becomes practicable. This is illustrated by the following example. Two fragments (N- and C-termini) of mitochondrial creatine kinase (Mi-CK) were biotechnologically produced in *E. coli*. Both fragments were successfully produced in large quantities by the bacteria and accumulated in the form of inclusion bodies. However, unlike the wild-type Mi-CK, the soluble form of the fragments is highly sensitive to proteolytic activity, a fact that rendered complete removal of any adhering proteases a prerequisite for successful purification of the renatured proteins.

Experimental design

Expression of the fragments took place over 5 hours at 37 °C. The *E. coli* cells were harvested by centrifugation, and washed with buffer P (PBS, pH 7.2 + 5 mM EDTA). The periplasmatic proteins were removed by swelling with distilled water on ice for 10 minutes followed by another centrifugation step. The cells were then lysed (on ice) with an ultrasonic probe in buffer containing only 1 U Benzonase® endonuclease per mL. The lysate was incubated for 30 minutes at 37 °C to digest the nucleic acids. Following centrifugation at 3,000 x g (15 minutes, 4 °C), the pellet, which contained the inclusion bodies as well as the *E. coli* cell fragments, was resuspended in buffer W (buffer + 25% sucrose + 1% Triton® X-100 + 1 U Benzonase® endonuclease per mL). It was then resubjected to ultrasonification and recentrifugation at 23,000 x g (10 minutes, 4 °C). The ultrasound/centrifugation procedure was then repeated twice. The inclusion bodies remain completely stable during this treatment. Excess detergent and Benzonase® endonuclease were then removed by washing twice with distilled water. The resulting inclusion body preparation proved to be very pure (see **Figure 15**). The inclusion bodies were dissolved in 8 M urea and, while still in a denatured state, subjected to one-step cation chromatography and purified to homogeneity.

SDS-polyacrylamide (15%) gel electrophoresis of N- and C-fragments Mi-CK
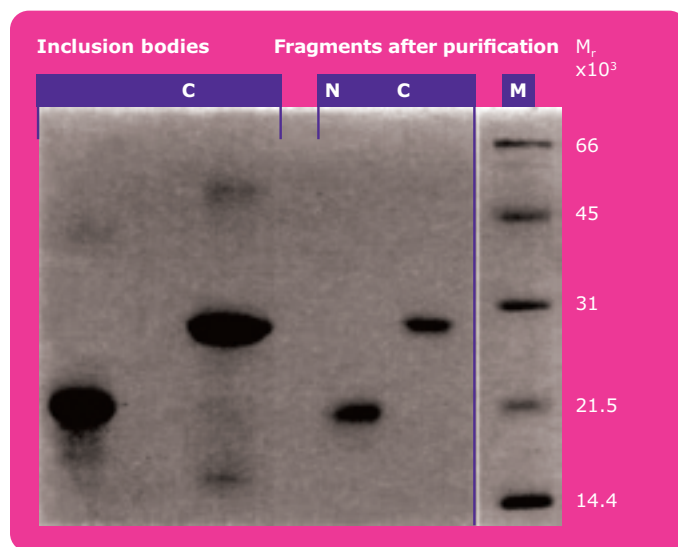

Figure 15.

Left: Ultrasound/Benzonase® endonuclease—treatment of washed inclusion bodies (strongly overloaded gel; practically no contamination visible).
Right: Renatured fragments after cation chromatography for purification and homogenization. Neither the raw inclusion body fraction nor the end product shows signs of the presence of proteolytic degradation products.

Results

When renatured by dilution or dialysis (no protease inhibitors were used) no proteolysis occurred; the purified fragments were 100% intact (see **Figure 15**) and resulted in enzymatically active Mi-CK when mixed *in vitro*. From a total of 1.6 L of bacterial culture, several hundred milligrams of both purified fragments could be obtained within two days (including both expression and purification). No proteolytic degradation of Mi-CK fragments was observed during refolding and purification. That indicates the successful and complete removal of any adhering proteases from the inclusion bodies.

Example 8: Use of Benzonase® endonuclease for sample preparation in two-dimensional gel electrophoresis

Two-dimensional (2-D) gel electrophoresis is a powerful technique for the high-resolution separation of complex mixtures of proteins. Nucleic acids are negatively charged molecules. They tend to form complexes by electrostatic interactions with positively charged domains on the surface of proteins. The formation and shape of these adducts usually cannot be predicted. These nucleic acid-protein complexes migrate differently in an electric field when compared to the pure protein. In addition to other effects, this may lead to band shifts in the expected protein pattern and cause poor resolution in 2-D gel electrophoresis. Sample pretreatment with Benzonase® endonuclease (50 U per 100 µL of cell lysate) strongly reduces horizontal streaking and significantly enhances the resolution of electrophoretic separation (**Figure 16**). It is important to note that the presence of Benzonase® endonuclease could not be detected on the gel due to the low amount of enzyme required.

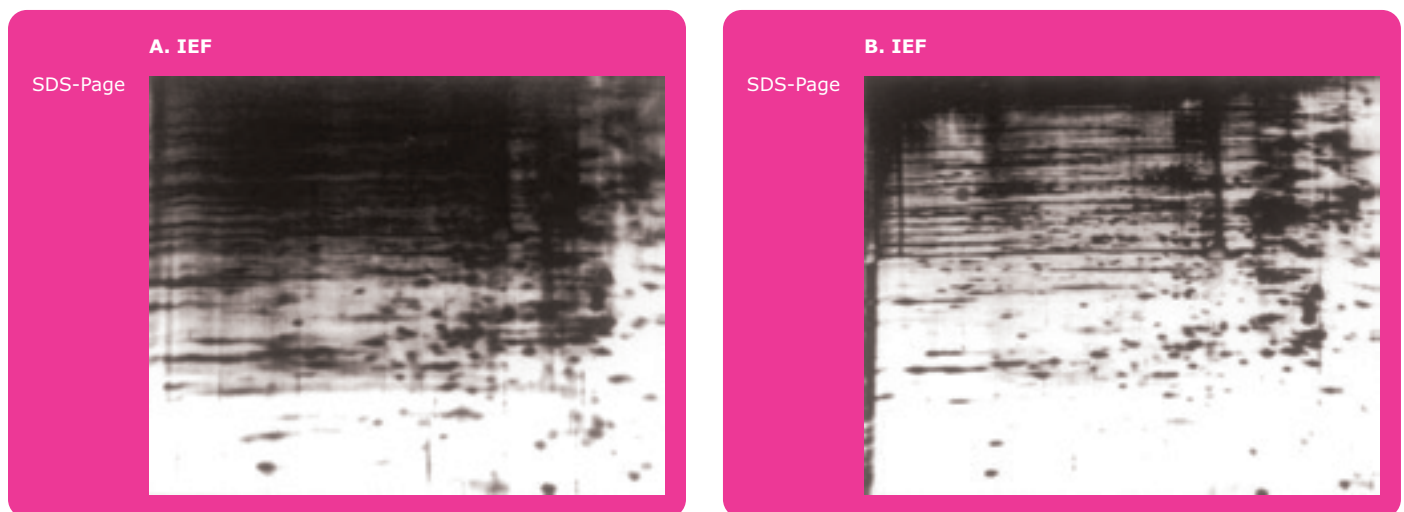**Silver-stained 2-D gel electrophoretic separation of bacterial cells (*Proteus vulgaris*)**

Figure 16.

A) Without use of Benzonase® endonuclease. **B)** By use of Benzonase® endonuclease.

Note: The presence of Benzonase® endonuclease could not be detected on the gel.

Frequently Asked Questions

Working with Benzonase® endonuclease for the first time or using it in a new application?

Consider this section your first stop when looking for answers. If you don't find what you're looking for here, please get in touch with your local Merck contact, or alternatively, see our website **MerckMillipore.com/benzonase**.

Which quality/quantity of Benzonase® endonuclease will be adequate for a certain application?

There are several parameters that influence the activity of Benzonase® endonuclease (see Benzonase® endonuclease triangle at right hand side). Hence, the optimum conditions will vary from process to process and need to be determined experimentally. For viscosity reduction, Benzonase® endonuclease, purity grade II (≥ 90%) will often be sufficient. Under standard assay conditions one unit of Benzonase® endonuclease corresponds approximately to the amount of enzyme required to completely digest 37 μg of DNA within 30 minutes.

Benzonase® endonuclease triangle

Incubation Time

Incubation Temperature

Quantity
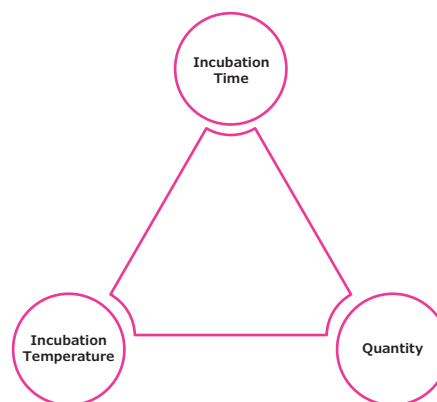
At which step do I have to introduce Benzonase® endonuclease in my process?

The answer to this question will vary depending on why you are using Benzonase® endonuclease. The example applications given will hopefully help you answer this question. However, as a general rule, Benzonase® endonuclease is usually best added after the cultivation and before the capture step.

How much more Benzonase® endonuclease do I have to add if I am working at low temperatures?

At temperatures below 37 °C, the efficiency of Benzonase® endonuclease decreases, as illustrated in the section on the temperature stability of Benzonase® endonuclease (see page 10). The amount needed to compensate for this decrease in efficiency will vary from process to process and depends on the other parameters present. Often, increasing another parameter, such as incubation time, can compensate without needing to increase the quantity of Benzonase® endonuclease used.

Why is Benzonase® endonuclease not working? What will inhibit its activity?

Benzonase® endonuclease is active under a wide range of operating conditions (see Enzyme Characteristics paragraph); however, a concentration of 1–2 mM Mg^{2+} is essential for the activity of Benzonase® endonuclease.

Mn^{2+} can substitute Mg^{2+}; however, the enzyme will only reach its optimum activity in the presence of Mg^{2+}. It is inhibited (approximately 50% activity) by monovalent cation concentrations > 300 mM, phosphate concentrations > 100 mM, and by ammonium sulfate concentrations > 100 mM. In addition, concentrations of > 1 mM EDTA will also inhibit Benzonase® endonuclease activity.

I observe a loss of activity: why?

Benzonase® endonuclease is usually very stable; however, in rare cases a loss of activity can be observed. There are several possible reasons for this: irreversible inactivation can be due to the presence of denaturing agents in the sample, e.g., proteases; or, alternatively, due to incorrect storage. Reversible inactivation is commonly due to the presence of chelating agents such as EDTA, which remove essential magnesium ions.

Is Benzonase endonuclease stable?

We have done extensive stability testing on Benzonase® endonuclease, and found that it is extremely stable. Even with extended incubations at 37 °C, Benzonase® endonuclease maintained > 90% of activity for several weeks. After a storage time of 11 weeks at 25 °C (60% RH), the activity even remained unchanged (see page 11, **Figure 2b**).

How do I inhibit Benzonase® endonuclease activity?

There are process additives/agents that affect Benzonase® endonuclease activity—for example, it can be inhibited by high salts, like > 300 mM monovalent cations, > 100 mM phosphate, > 100 mM ammonium sulfate, > 100 mM guanidine HCl. Other known inhibitors are chelating agents, like EDTA, which could cause loss of free Mg^{2+}-ions (EDTA concentrations > 1 mM have shown to inhibit the enzymatic reaction). This can be reversed by adding more $MgCl_2$.

How do I remove Benzonase® endonuclease?

Removal of Benzonase® endonuclease can be accomplished by several downstream units of operation, like depth filtration for clarification, tangential flow filtration (TFF) for concentration and diafiltration and chromatography (IEX, SEC, HIC). Please see Appendix, Chapter 2 "Removal of Benzonase® endonuclease" (page 36) for further information.

Is Benzonase® endonuclease safe?

Yes, toxicological studies with Benzonase® endonuclease have been performed (internal reports available). The systemic toxicity after single application was investigated in mice and rats: no toxic effects have been observed even at very high doses. In addition, no mutagenic potential has been observed in mice treated intravenously even with a very high dose of Benzonase® endonuclease.

Is Benzonase® endonuclease free of protease activity?

Yes, Benzonase® endonuclease is supplied without detectable protease activity and is hence not degraded during its "work." The presence of protease in the sample itself will, however, result in irreversible degradation of the Benzonase® endonuclease.

Is Benzonase® endonuclease compatible with protease inhibitor cocktails?

Yes. However, caution should be exercised, since many protease inhibitor cocktails include EDTA. Concentrations of greater than 1 mM EDTA will inhibit the activity of Benzonase® endonuclease.

Do you offer immobilized Benzonase® endonuclease?

No. All efforts to bind Benzonase® endonuclease to a support that meets the demands of a commercial product with respect to activity, stability and regulatory requirements have so far been unsuccessful.

Why is the filling range volume of the 5-million-unit tubes not specified?

As the activity (U/mL) of Benzonase® endonuclease may vary between production lots, we decided to specify the units per tube, but not the volume.

References

Elimination of nucleic acids from recombinant proteins

Ye, G.-J., et al. (2011) Clearance and characterization of residual HSV DNA in recombinant adeno-associated virus produced by an HSV complementation system. *Gene Ther.* **18**, 135.

Shi, H., et al. (2009) Mitogen-activated protein kinase signaling is necessary for the maintenance of skeletal muscle mass. *Am. J. Physiol. Cell Physiol.* **296**, C1040.

Quinlivan, E. P., et al. (2008) DNA digestion to desoxyribonucleoside: A simplified one-step procedure. *Anal. Biochem.* **373**, 383.

Tang, R., et al. (2008) Development of a PIV-vectored RSV vaccine: Preclinical evaluation of safety, toxicity, and enhanced disease and initial clinical testing in healthy adults. *Vaccine* **26**, 6373.

Transfiguracion, J., et al. (2007) High yield purification of functional baculovirus vectors by size exclusion chromatography. *J. Virol. Methods.* **142**, 21.

Hervas-Stubbs, S., et al. (2007) Insect baculoviruses strongly potentiate adaptive immune responses by inducing type I IFN. *J. Immunol.* **178**, 2361.

Kercher, L., et al. (2007) Prion protein expression differences in microglia and astroglia influence scrapie-induced neurodegeneration in retina and brain of transgenic mice. *J. Virol.* **81**, 10340.

Cho, S., et al. (2005) Glucocorticoid receptor ligand binding domain is sufficient for the modulation of glucocorticoid induction properties by homologous receptors, coactivator transcription intermediary factor 2, and Ubc9. *Mol. Endocrinol.* **19**, 290.

Han, W., et al. (2005) C-terminal ECFP fusion impairs synaptotagmin 1 function: crowding out synaptotagmin 1. *J. Biol. Chem.* **280**, 5089.

Meyn, M., et al. (2005) Src family kinase activity is required for murine embryonic stem cell growth and differentiation. *Mol. Pharmacol.* **68**, 1320.

Murphy, M., et al. (2003) An improved method for the *in vitro* evolution of aptamers and applications in protein detection and purification. *Nucleic Acids Res.* **31**, e110.

Persad, S., et al. (2001) Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase. *J. Bio. Chem.* **276**, 27462.

Viral Vaccine and Viral Vector Applications

Meeting Report (2007) – WHO Study Group on Cell Substrates for Production of Biologicals, WHO Headquarters, Geneva, 11–12 June 2007

World Health Organization (2010) Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks Proposed replacement of TRS 878, Annex 1

U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research (2010) Guidance for Industry Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications

Patent WO1994003589 A2

Mundle, S, et al. (2013) High-Purity Preparation of HSV-2 Vaccine Candidate ACAM529 Is Immunogenic and Efficacious In Vivo. PLoS ONE **8**(2): e57224.

Milián, E, et al. (2015) Current and Emerging Cell Culture Manufacturing Technologies for Influenza Vaccines. BioMed Research International., 2015:504831.

Josefsberg, J, et al. (2012) Vaccine Process Technology. Biotechnology and Bioengineering, Vol. **109**, No. 6

Cook, J, et al. (1999) Purification of Virus-like Particles of Recombinant Human Papillomavirus Type 11 Major Capsid Protein L1 from Saccharomyces cerevisiae. Protein Expr Purif., **17**(3):477-84

van de Waterbeemd, B, et al. (2013) Improved Production Process for Native Outer Membrane Vesicle Vaccine against Neisseria meningitidis. PLoS ONE **8**(5): e65157.

Pajon, R, et al. (2011) Meningococcal Factor H Binding Proteins in Epidemic Strains from Africa: Implications for Vaccine Development. PLoS Negl Trop Dis **5**(9): e1302.

Bauer, S, et al. (2002) Testing of adenoviral vector gene transfer products: FDA Expectations. Adenoviral Vectors for Gene Therapy; Academic Press: New York, pp 615-654.

Green, A, et al. (2001) A new scalable method for the purification of recombinant adenovirus vectors. Hum Gene Ther, **13** (16): 1921-1934.

Clément, N and Grieger, J, (2016) Manufacturing of recombinant adeno-associated viral vectors for clinical trials. Molecular Therapy—Methods & Clinical Development **3**, 16002

Ausubel, L, et al. (2012) Production of CGMP-Grade Lentiviral Vectors. Bioprocess Int., 10(2): 32–43.

Isayeva, T, et al. (2003) Advanced Methods of Adenovirus Vector Production for Human Gene Therapy: Roller Bottles, Microcarriers, and Hollow Fibers. BioProcess J, **2**(5): 75-81.

Slepushkin, V, et al. (2003) Large-scale Purification of a Lentiviral Vector by Size Exclusion Chromatography or Mustang Q Ion Exchange Capsule. BioProcess J, **2**(5): 89-95.

Enhanced protein purification via complete cell lysis and viscosity reduction

Collin, R., et. al. (2009) The DEXD/H-box RNA Helicase DDX19 is regulated by an α-helical switch. *J. Biol. Chem.* **284**, 10296.

Gustin, K., et al. (2009) Bovine coronavirus nonstructural protein 1(p28) is an RNA binding protein that binds terminal genomic cis-replication elements. *J. Virol.* **83**, 6087.

Wanyiri, J., et al. (2009) Role CpSUBI, a subtillisin-like protease, in *Cryptosporidium parvum* infection *in vitro*. *Eukaryot. Cell.* **8**, 470.

Shen, X., et al. (2009) Identification of novel SHPS-I-associated proteins and their roles in regulation of insulin-like growth factors-dependent responses in vascular smooth muscle cells. *Mol. Cell. Proteomics* **8**, 1539.

Heikkilä, O., et al. (2009) Integrin vβ6 is a high-affinity receptor for coxsackievirus A9. *J. Gen. Virol.* **90**, 197.

Masip, L., et al. (2008) Laboratory evolution of *Escherichia coli* thioredoxin for enhanced catalysis of protein oxidation in the periplasm reveals a phylogenetically conserved substrate specificity determinant. *J. Bio. Chem.* **283**, 840.

Suzuki, C., et al. (2008) PDCD4 inhibits translation initiation by binding to eIF4A using both its MA3 domains. *Proc. Natl. Acad. Sci.* **105**, 3274.

Lloyd-Burton, S., et al. (2007) Regulation of inositol 1,4,5-triphosphate 3-kinases by calcium and localization in cells. *J. Biol. Chem.* **282**, 9526.

Park, M., et al. (2007) Glyceraldehyde-3-phosphate ferredoxin oxidoreductase from *Methanococcus maripaludis*. *J. Bacteriol.* **189**, 7281.

Kobayashi, S., et al. (2007) Serine 64 phosphorylation enhances the antiapoptotic function of Mcl-1. *J. Biol. Chem.* **282**, 18407.

Miyazaki, K., et al. (2006) Thermal stabilization of *Bacillus subtilis* family-11 xylanase by directed evolution. *J. Biol. Chem*. **281**, 10236.

Rangarajan, E., et al. (2005) Crystallographic trapping of the glutamyl-CoA thioester intermediate of family I CoA transferases. *J. Biol. Chem.* **280**, 42919.

Frankel, P., et al. (2005) RalA interacts with ZONAB in a cell density-dependent manner and regulates its transcriptional activity. *Eur. Mol. Bio. Org. J.* **24**, 54.

Johnsen, L., et al. (2005) 1.6 Å crystal structure of EntA-im: A bacterial immunity protein conferring immunity to the anti-microbial activity of the pediocin-like bacteriocin enteroc in A. *J. Biol. Chem.* **280**, 19045.

Kumaran, D., et al. (2005) Structure and mechanism of ADP-ribose-1''-monophosphatase (Appr-1''-pase), a ubiquitous cellular processing enzyme. *Protein. Sci.* **14**, 719.

Li, L., et al. (2005) Clarification of cinnamoyl co-enzyme A reductase catalysis in monolignol biosynthesis of Aspen. *Plant Cell Physiol.* **46**, 1073.

Yamakoshi, Y., et al. (2005) Porcine dentin sialoprotein is a proteoglycan with glycosaminoglycan chains containing chondroitin 6-sulfate. *J. Biol. Chem.* **280**, 1552.

Zanghi, C., et al. (2005) A simple method for displaying recalcitrant proteins on the surface of bacteriophage lambda. *Nucleic Acids Res.* **33**, e160.

Kasim, V., et al. (2004) Control of siRNA expression using the Cre-loxP recombination system. *Nucleic Acids Res.* **32**, e66.

Lunin, V., et al. (2004) The structure of the MAPK scaffold, MP1, bound to its partner, p14. A complex with a critical role in endosomal MAP kinase signaling. *J. Biol. Chem.* **279**, 23422.

Rizzo, M., et al. (2004) An improved cyan fluorescent protein variant useful for FRET. *Nat. Biotechnol.* **22**, 445.

Chan, W., et al. (2002) Human phosphatidylcholine transfer protein: purification, crystallization and preliminary X-ray diffraction data. *Biochim. Biophys Acta* **1596**, 1.

Huhtinen, K., et al. (2002) The peroxisome proliferatorinduced cytosolic type I acyl-CoA thioesterase (CTE-I) is a serine-histidine-aspartic acid /βhydrolase. *J. Biol. Chem.* **277**, 3424.

Lobel, L., et al. (2002) Bacterial expression of a natively folded extracellular domain fusion protein of the hFSH receptor in the cytoplasm of *Escherichia coli*. *Protein Expr. Purif.* **25**, 124.

Peitz, M., et al. (2002) Ability of the hydrophobic FGF and basic TAT peptides to promote cellular uptake of recombinant Cre recombinase: a tool for efficient genetic engineering of mammalian genomes. *Proc. Natl. Acad. Sci.* **99**, 4489.

Suarez, T., et al. (2002) Biochemical defects in retina-specific human ATP binding cassette transporter nucleotide binding domain 1 mutants associated with macular degeneration. *J. Biol. Chem.* **277**, 21759.

Glick, E., et al. (2001) Mutations in human DNA polymerase η motif II after bypass of DNA lesions. *Eur. Mol. Bio. Org. J.* **20**, 7303.

Lobel, L., et al. (2001) High-level bacterial expression of a natively folded, soluble extracellular domain fusion protein of the human luteinizing hormone/chorionic gonadotropin receptor in the cytoplasm of *Escherichia coli*. *Endocrine* **14**, 205.

Sample preparation in gel electrophoresis

Milovnik, P., et. al. (2009) Selection and characterization of DARPins specific for the neurotensin receptor I. *Protein Eng. Des. Sel.* **22**, 357.

Oliveira, J., et al. (2007) NRAS mutation causes a human autoimmune lymphoproliferative syndrome. *Proc. Natl. Acad. Sci.* **104**, 8953.

Kwade, Z., et al. (2005) Identification of four adenosine kinase isoforms in tobacco By-2 cells and their putative role in the cell cycle regulated cytokinin metabolism. *J. Biol. Chem.* **280**, 17512.

Poirier, M., et al. (2005) A structure-based analysis of huntingtin mutant polyglutamine aggregation and toxicity: evidence for a compact beta-sheet structure. *Human Mol. Genet.* **14**, 765.

Mercer, K.L.N. et al. (2002) The *Escherichia coli* cell division protein FtsW is required to recruit its cognate transpeptidase, FtsI (PBP3), to the division site. *J. Bacteriol.* **184**, 904.

Prevention of cell clumping

Doria-Rose, N., et. al. (2009) Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies. *J. Virol.* **83**, 188.

Huaman, M., et al. (2008) "Ex vivo cytokine and memory T cell responses to the 42-kDa fragment of *Plasmodium falciparum* merozoite surface protein-1 in vaccinated volunteers." *J. Immunol.* **180**, 1451.

Mingozzi, F., et al. (2007) Modulation of tolerance to the transgene product in a nonhuman primate model of AAV-mediated gene transfer to liver. *Blood* **110**, 2334.

Keating, S., et al. (2005) Durable human memory T cells quantifiable by cultured enzyme-linked immunospot assays are induced by heterologous prime boost immunization and correlate with protection against malaria. *J. Immunol.* **175**, 5675.

Vuola, J., et al. (2005) Differential immunogenicity of various heterologous prime-boost vaccine regimens using DNA and viral vectors in healthy volunteers. *J. Immunol.* **174**, 449.

Ordering Information

Benzonase® endonuclease is manufactured in Denmark. It is distributed worldwide exclusively by Merck KGaA, Darmstadt, Germany its affiliates, and selected dealers. Benzonase® endonuclease is supplied in 20 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 20 mM NaCl, and 50% (v/v) glycerol in three different package sizes and two purity grades.

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 +50,000 U	**1.03773.0010**
Benzonase® endonuclease ELISA Kit III for the immunological detection of Benzonase® endonuclease	5 plates (8 x 12) 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)

Appendix

1. Benzonase® endonuclease Standard Activity Assay (volume activity)

Calculation principle

Benzonase® endonuclease degrades sonicated DNA into oligonucleotides of three to five base pairs in length. The production of these oligonucleotides leads to an increase in absorbance at 260 nm.

Unit definition

One unit corresponds to the amount of enzyme required to produce a change in absorbance at 260 nm of 1.0 in the time of 30 minutes, under optimum conditions with excess substrate.

Reagents and solutions

1. Reagent A (1 mM MgCl$_2$, 0.1 mg/mL BSA, in 50 mM Tris, pH 8.0)

Dissolve 3.0 g Tris (Cat. No. 648310) in about 480 mL of redistilled water, adjust to pH 8.0 with 1.0 mol/l hydrochloric acid (Cat. No. 1.09057), and make up to 500 mL with redistilled water (results in 0.05 mol/l Tris/HCl buffer solution). Subsequently add 0.10 g of magnesium chloride hexahydrate (Cat. No. 1.05833) and 50 mg of albumin fraction V (Cat. No. 1.12018).

2. Reagent B (0.1 mg/mL DNA)

Dissolve herring sperm DNA (Cat. No. D1815, Promega) in reagent A to a final concentration of 0.1 mg/mL.

3. Perchloric acid solution (4%)

Dilute 5.63 mL 70–72% perchloric acid (Cat. No. 1.00519) with redistilled water to 100.0 mL.

4. Enzyme (Benzonase® endonuclease) solution

Dilute 16 µL of the sample with ice-cold reagent A to 100.0 mL and make up 10.0 mL of this solution with reagent A to 50.0 mL.

Assay Procedure

We recommend to perform each test series in duplicate along with a blank value.
The incubation of the test samples should be performed in centrifugation tubes.

	Sample	Blank value
Reagent B	2.500 mL	2.500 mL
Enzyme solution	0.125 mL	–
Reagent A	–	0.125 mL
Incubate in a water bath at 37 °C		

After 15 min, 30 min, 45 min and 60 min, pipette 0.500 mL each of the incubation preparation into an Eppendorf tube (1.4 mL) containing 0.500 mL of perchloric acid (3). Mix, incubate on ice 30 to 60 min, and centrifuge at 14,000 rpm, for 6 min at 4 °C. Transfer the supernatant into new Eppendorf tubes and measure the absorbance of the samples against the blank.

Wavelength: 260 nm	**Optical path length: 1 cm**

Calculation

$$U/\mu L = \frac{\Delta A \cdot 30 \cdot V \cdot 2 \cdot F}{t \cdot v \cdot 1000}$$

➡

$$U/\mu L = \frac{\Delta A \cdot 30 \cdot 2.625 \cdot 2 \cdot 31250}{t \cdot v \cdot 1000}$$

➡

$$U/\mu L = \frac{\Delta A \cdot 39375}{t}$$

where

A = absorbance of the measuring solution at time t

V = total volume of the incubation mixture

F = dilution factor sample solution

t = incubation time of the measuring solution

v = sample volume

30 = one unit is defined at 30 min

2 = dilution factor of the measuring solution

1000 = conversion factor of mL to µL

2. Removal of Benzonase® endonuclease

Removal of Benzonase® endonuclease from the manufacturing process can be accomplished in several ways during downstream purification operations, such as tangential flow filtration (TFF) or chromatography. Successful removal of Benzonase® endonuclease can be shown by demonstrating no residual nuclease activity (does not detect residual non-active Benzonase® endonuclease) and through ELISA for detection of the total residual endonuclease (active and non-active). Drug product manufacturers using Benzonase® endonuclease in the manufacturing process of their drug need to demonstrate data related to its safety/toxicity and measure residual endonuclease that may be present in the final preparation.

Benzonase® endonuclease is easily removed with the filtrate while the drug product can be retained by using TFF and by selection of appropriate membrane cut-offs. Benzonase® endonuclease can be removed from the process by diafiltration to concentrations below detectable limit (**Figure 17**).

Depending on purification challenges, ion-exchange chromatography may be used alternatively or additionally to TFF for removal of Benzonase® endonuclease. Ion chromatography is well-known for removal of residual endonuclease from drug products. With a pI of 6.85, it will typically flow through anion exchange chromatography (AEX) while the drug product may be bound to the AEX column, or in case it is bound as well, elutes separately. Several anion exchange resins using a variety of sample and equilibration buffers are illustrated for Benzonase® endonuclease removal.

Cation exchange chromatography (CEX) can also be effective in removal of Benzonase® endonuclease, but the operating range might be smaller. Subsequent tables provide a reference list for a few chromatography media and conditions that are suitable for the removal of Benzonase® endonuclease.
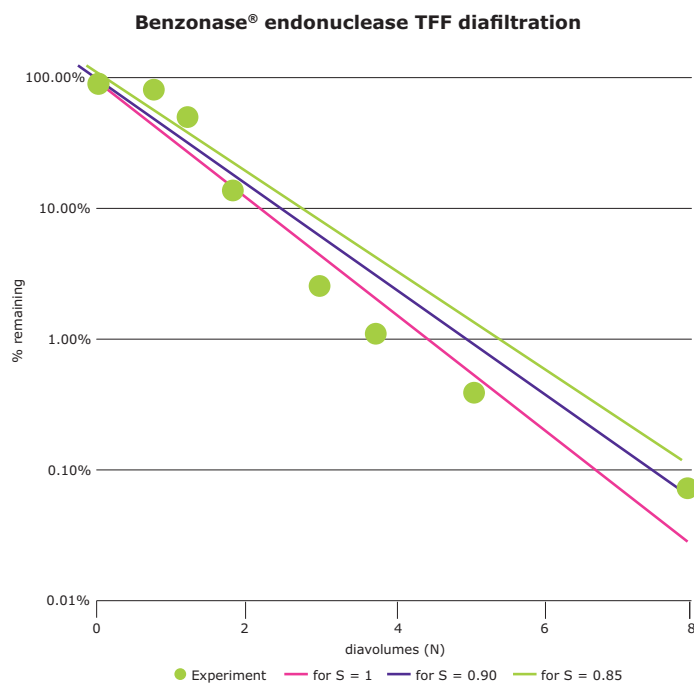

Figure 17.

TFF diafiltration with the example of a viral vaccine production process: up to > 99.5% clearance of Benzonase® endonuclease at 5 diavolumes and > 99.9% after 8 diavolumes using a 300 kD membrane. The overall diafiltration profile follows close to a theoretical sieving value of 1.

Anion exchange chromatography for removal of Benzonase® endonuclease

Fractogel® EMD resin	pH	Sample & equilibration buffer	Benzonase® endonuclease	BSA
TMAE	7.0	50 mM Tris / 200 mM NaCl	not bound	not bound
TMAE	7.0	50 mM Tris / 50 mM NaCl	not bound	bound
TMAE	8.0	50 mM Tris / 250 mM NaCl	not bound	not bound
TMAE	8.0	50 mM Tris / 100 mM NaCl	not bound	bound
TMAE	9.0	50 mM Tris / 200 mM NaCl	not bound	partially bound
TMAE	9.0	50 mM Tris / 100 mM NaCl	not bound	bound
DEAE	7.0	50 mM Tris / 200 mM NaCl	not bound	not bound
DEAE	7.0	50 mM Tris / 50 mM NaCl	not bound	bound
DEAE	8.0	50 mM Tris / 250 mM NaCl	not bound	not bound
DEAE	8.0	50 mM Tris / 100 mM NaCl	not bound	bound
DEAE	9.0	50 mM Tris / 250 mM NaCl	not bound	not bound
DEAE	9.0	50 mM Tris / 50 mM NaCl	not bound	bound
DMAE	8.0	50 mM Tris / 250 mM NaCl	not bound	partially bound
DMAE	8.0	50 mM Tris / 50 mM NaCl	not bound	bound

TMAE = Trimethylammoniumethyl; DEAE = Diethylaminoethyl; DMAE = Dimethylaminoethyl

Cation exchange chromatography for removal of Benzonase® endonuclease

Fractogel® EMD resin	pH	Sample and equilibration buffer	Benzonase® endonuclease
SO_3^-	6.0	20 mM phosphate / 100 mM NaCl	bound
SO_3^-	6.0	20 mM phosphate / 200 mM NaCl	not bound
SO_3^-	5.0	20 mM acetate / 200 mM NaCl	bound
SO_3^-	5.0	20 mM acetate / 700 mM NaCl	not bound
SO_3^-	4.0	20 mM acetate / 300 mM NaCl	bound
SO_3^-	4.0	20 mM acetate / 800 mM NaCl	not bound
COO^-	6.0	20 mM phosphate / 0 mM NaCl	not bound
COO^-	5.0	20 mM acetate / 40 mM NaCl	bound
COO^-	5.0	20 mM acetate / 100 mM NaCl	not bound
COO^-	4.0	20 mM acetate / 150 mM NaCl	partially bound
COO^-	4.0	20 mM acetate/ 400 mM NaCl	not bound

Gousseinov, E., et al. (2013). Nucleic Acid Impurity Reduction in Viral Vaccine Manufacture. *Bioprocess Int*. in press.
Eglon, M., et al. (2009). Purification of adenoviral vectors by combined anion exchange and gel filtration chromatography. *J. Gene Med*. 11, 978.

3. Detection of Benzonase® endonuclease—
Benzonase® ELISA Kit III is now available (104358 & EZBNZ3-160K)

This assay detects and quantifies the amount of Benzonase® endonuclease present in samples, thus allowing proof of its removal. The use of purified polyclonal antibodies specific to Benzonase® endonuclease results in a precise assay with a sensitivity of 32 pg/mL. Cross-reactions with standards cell culture supernatant are less than 1%.
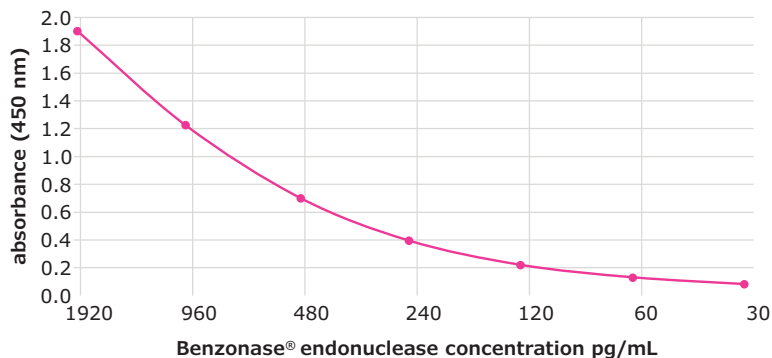

Figure 1. Example of a typical calibration curve for Benzonase® endonuclease, purity grade I (≥ 99%) in the range of 31 to 2000 pg/mL Benzonase® endonuclease concentration absorbance (450 nm).

How does the assay work?

The assay uses purified polyclonal antibodies specific to Benzonase® endonuclease, which have been coated on to polystyrene microtiter plates. After samples are added, Benzonase® endonuclease is captured on the plates.

After further addition of biotin-conjugated antibodies specific to Benzonase® endonuclease, Streptavidin-POD (Peroxidase) and TMB (3, 3, 5, 5'-tetramethyl-benzidine), the reaction is stopped by the addition of 0.2 M H_2SO_4. Result can be read using an ELISA plate reader at 450 nm. Visual read is not recommended.
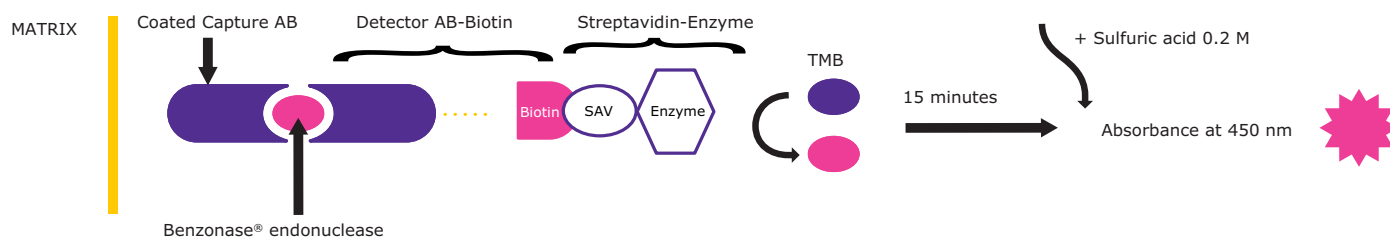

Figure 19. Schematic drawing of the ELISA working principle used for Benzonase® endonuclease quantification.

**104358, or EZBNZ3-160K
two options for a full supply
robustness**

Dual source available

Key Benefits

- Fast and simple to use
- High sensitivity (32 pg/mL)
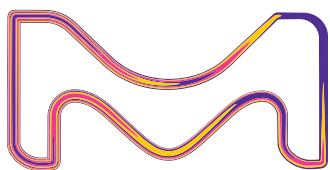- Single strip plates (8 x 12)
- MQ300

SAFC®

Pharma & Biopharma Raw
Material Solutions

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

MerckMillipore.com