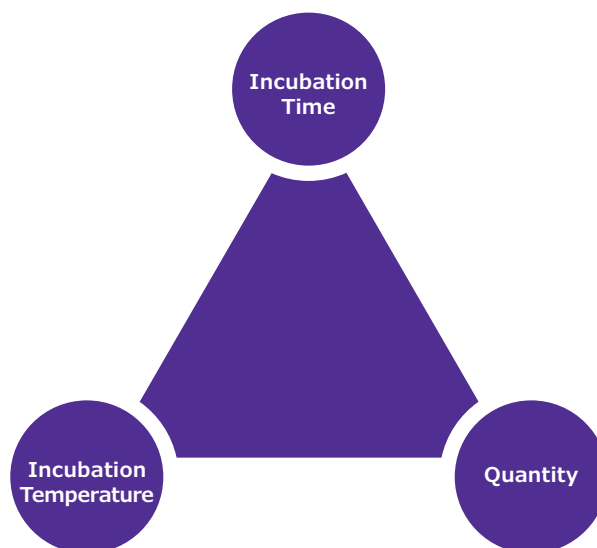




# Optimization of Benzonase® endonuclease use in virus purification

Benzonase® endonuclease— the smart solution for DNA removal in biopharmaceutical production has proven its value for over 30 years. Balancing efficiency and regulatory compliance by delivering reliability and high-quality due to manufacturing under GMP (ICH Q7). All Benzonase® endonuclease products share the same amino acid sequence (proven by LC-MS/MS mass spectrometry), robustness and activity. Acting as endonuclease, degrading both DNA and RNA to small 3–5 base pairs (<6 kDa) fragments with no base preference, they are the ideal tool for nucleic acid removal in virus vector and vaccine manufacturing as demanded by the regulators. The use of Benzonase® endonuclease additionally increases the yield in virus purification, protects the downstream chromatography and filter devices from fouling and reduces feed stream viscosity.

## Benzonase® endonuclease triangle



### Introduction into DoE

Design of experiments (DoE) is employing statistics to plan, conduct, and analyze the effect of multiple factors on a parameter or group of parameters. DoE is a powerful tool, that can be used in a variety of experimental situations, such as the optimization of enzymatic reaction conditions. Setting up a DoE for your Benzonase® endonuclease application can help you to find the optimal operation conditions that deliver the required DNA clearance from your process.

### Why optimizing Benzonase® endonuclease use in your manufacturing process

Benzonase® endonuclease is a high quality product delivering big value to your process. Using it in the most economic way is key. As Benzonase® endonuclease activity is strongly influenced by the matrix it is applied in optimization of its use should be a mandatory and crucial step in process development.

In this document we will provide you guidance on how to plan and set up a Design of Experiments (DoE) optimization experiment of Benzonase® endonuclease.

Please note that optimization of Benzonase® endonuclease point of use (e.g. in the bioreactor, post cell lysis or after clarification unit operation.) is not covered in this document.

## Center point starting conditions for the defined variables

We recommend to set the starting point as follows:

- Incubation time set to 2 hours (according to table)
- Incubation temperature set to room temperature e.g. 21°C (according to table)
- Benzonase® endonuclease theoretical concentration under standard assay conditions, according to the following formula, based on the DNA load of respective sample material:

$$\frac{\text{DNA in sample } [\mu\text{g/mL}]}{37} = \frac{\text{Units Benzonase}^{\circledR} \text{ endonuclease}}{\text{mL of process volume}}$$

### Dosage of Benzonase® endonuclease into respective unit operation

Benzonase® endonuclease dosage should be based on the actual activity in U/μL, given on the COA. Applying the enzyme by activity, instead of protein concentration, ensures reproducible DNA digestion.

## Setting up a DoE

To set up an experiment series that follows the concept of DoE it is recommended to define constant factors, input variables with center point starting conditions and output parameters, first.

### Definition of constant factors

- The average DNA load of the respective sample material should be known to calculate the required amount of enzyme
- As the composition of the material from the upstream process of viral manufacturing is complex, varying between applications, but consistent for each targeted optimization of Benzonase® endonuclease, it can be considered as a constant factor as well as the resulting pH and salt concentrations. For an overview of the effect of different chemicals on Benzonase® endonuclease performance please also see our product brochure.
- As Mg<sup>2+</sup> concentration is the most critical parameter for Benzonase® endonuclease functionality it should always be kept constant at 2 mM
- Incubation vessel and mixing process should be kept constant

### Definition of input variables

- In the outlined DoE three different values per variable are recommended, representing upper- (pattern symbol +), center- (pattern symbol 0), and low value (pattern symbol -)
- Balancing the performance of Benzonase® endonuclease depends on:
  - Benzonase concentration
  - Incubation time
  - Incubation temperature

### Definition of output

- As the goal of Benzonase® endonuclease application is DNA digestion the key outputs are:
  - DNA content of >100 bp fragments
  - Product integrity

### Measuring Benzonase® endonuclease performance:

DNA digestion by Benzonase® endonuclease should be monitored using a specific qPCR method for a 100-200 base pair fragment of host cell DNA. Complete DNA digestion by Benzonase® endonuclease will result in 3-5 base pair fragments, which limits the utility of fluorescent DNA-intercalating dye assays that detect DNA fragments as small as 4 base pairs. We recommend performing appropriate control experiments to define the digestion process, therefore ensuring robust results.

Pattern +: Upper Value 0: Center Value -: Low Value	Process Parameters		
	Benzonase® endonuclease Concentration (x Theoretical Concentration)	Incubation Time(h)	Temperature (°C)
000	1	2	21
-++	0.5	6	37
+--+	10	1	37
---	0.5	1	4
+--	10	1	4
++-	10	6	4
+++	10	6	37
0++	1	6	37
+0+	10	2	37
0+0	1	6	21
++0	10	6	21
0--	1	1	4
0-0	1	1	21
-0-	0.5	2	4
--0	0.5	1	21

## Interpretation of the DoE Results and next steps

The experimental design proposed in the table is a suggestion based on the main parameters that influence DNA/RNA digestion. It is possible to reduce the number of trials depending on your current resources or add more conditions. Once the design has been executed, it is recommended to first verify, that the data generated are consistent with the initial experimental assumptions. The results are then fully analyzed and interpreted. This may lead to further experiments or an additional DoE with more narrowed parameter values around the determined optimum. Additionally the optimization of the point of use in the manufacturing process such as in the bioreactor, post cell lysis or after clarification should be considered to deliver the most economic performance of Benzonase® endonuclease.

MilliporeSigma  
400 Summit Drive  
Burlington, MA 01803

## To place an order or receive technical assistance

In the U.S. and Canada, call toll-free 1-800-645-5476

For other countries across Europe and the world, please visit: [EMDMillipore.com/offices](https://www.emdmillipore.com/offices)

For Technical Service, please visit: [EMDMillipore.com/techservice](https://www.emdmillipore.com/techservice)

[EMDMillipore.com](https://www.emdmillipore.com)

