

Gyrolab® Assay Protocol

Benzonase® endonuclease Assay

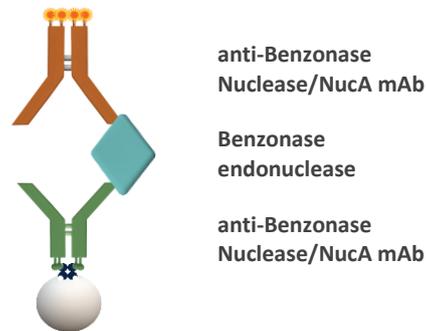
INTRODUCTION

Benzonase® endonuclease (registered trademark of Merck KGaA) is a genetically engineered enzyme from *Serratia marcescens* that can be used to degrade all forms of nucleic acids and is active over a range of operating conditions. Benzonase is commonly used to remove nucleic acids during production of recombinant proteins and must be subsequently removed in downstream purification. It has also been used to limit cell clumping during preparation of cell mixtures.

We have developed a three-step sandwich Gyrolab® assay to quantify Benzonase nuclease impurities in bioprocess samples. The assay has a broad analytical range with an approximate LOD of 0.2 ng/mL, LLOQ of 0.3 ng/mL, and ULOQ of 225 ng/mL. Use of this protocol on Gyrolab systems will reduce time to market and increase productivity while maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a three-step sandwich assay with biotinylated anti-Benzonase Nuclease/NucA antibody as a capture molecule and a different anti-Benzonase Nuclease/NucA antibody labeled with Alexa Fluor® 647 as a detection molecule. The assay standard used was the Benzonase endonuclease from Merck¹.



ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 4-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 0.3 ng/mL to 225 ng/mL (Table 1). The Limit of Detection (LOD) was determined as the concentration corresponding to at least two standards deviations above the assay blank.

The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in triplicates in three runs, was <20% (Table 2).

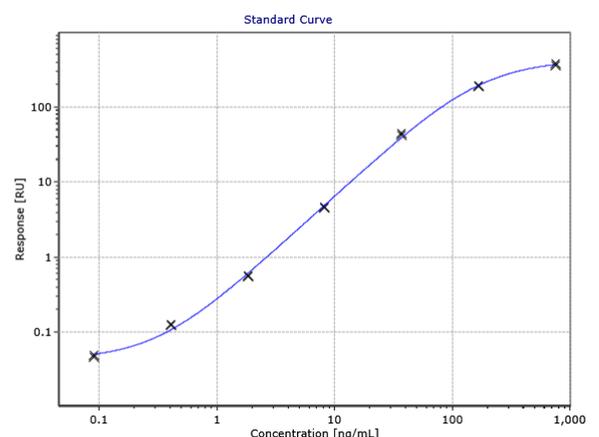


Figure 1 Typical standard curve in REXXIP® A

Table 1 Estimated Assay Range in based on three runs

LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
~ 0.2	~ 0.3	~ 225

¹ For the product range Benzonase, the supplier does not indicate the concentration in mg/mL, but in U/mL instead, considering that the activity (U/mL) of Benzonase endonuclease may vary between production lots. According to the Certificate of Analysis, the specific batch of Benzonase endonuclease that was used had an activity of 358 U/μL and a specific activity of 1.9*10⁵ U/mg. The activity was converted to concentration units corresponding to 0.1884 mg/mL.

Table 2 Accuracy and precision data of QC samples, n = number of runs

QC	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	Inter Run CV (%; n=3)	Average Intra Run CV (%; n=3)	Average Total Error (%; n=3)
LLOQ	0.3	0.34	16	10	23
LQC	0.9	1.0	6.6	5.9	14
MQC	15	15	3.2	3.0	6.3
HQC	150	153	4.2	3.9	6.3
ULOQ	225	210	5.7	5.2	12

MATERIALS AND METHODS

The assay was developed on a Gyrolab system using Gyrolab Bioaffy™ 4000 CD. The assay was set up using a three-step method with two wash-solutions (4000-3W-001-A) and a 1% PMT setting. The assay buffer was Rexpip A. Rabbit monoclonal anti-Benzonase Nuclease/NucA antibody (clone 2404B) from R&D Systems was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 100 µg/mL, diluted with PBS-T.

The detection antibody, labeled with Alexa Fluor 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the rabbit monoclonal anti-Benzonase Nuclease/NucA antibody (clone 2404D) from R&D Systems, diluted to 50 nM in Rexpip F. The assay standard used was Benzonase endonuclease (catalogue no. 101695 from Merck). The standard and controls were prepared in Rexpip A.

Summary table

Capture	Rat monoclonal anti-Benzonase Nuclease/NucA antibody (clone 2404B, R&D Systems, MAB10063), biotinylated and diluted to 100 µg/mL in PBS-T
Detection	Rat monoclonal anti-Benzonase Nuclease/NucA antibody (clone 2404D, R&D Systems, MAB100632) labeled with Alexa Fluor 647, 50 nM in Rexpip F
Analyte	Benzonase endonuclease EMPROVE® Expert (Merck KGaA, 101695) in Rexpip A
CD-type	Gyrolab Bioaffy 4000
Method	4000-3W-001-A
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11
PMT-setting	1%
Expected dynamic range	Approximately 0.3 ng/mL–225 ng/mL

Recommendations

When developing this assay for a specific bioprocess, it is important to screen matrices and assess backgrounds. Parameters, such as LLOQ, should be validated in-house. Data given in this document should only be considered as guidance.

For additional support contact your local Field Application Support

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