

THE KEY ADVANTAGE TO USE FASTNUCLEASE™ IN YOUR PROCESSES IS THE AVAILABILITY OF AN ASSAY SPECIFIC TO FASTNUCLEASE™ TO MEASURE FOR ANY RESIDUAL IMPURITIES LEFT OVER IN YOUR PRODUCT / PROCESS / CELL CULTURE.

## Protein Modifying Enzymes Available with us

Cat No	Product Particulars	Pack Size
PROTEASE		
KBENZ45	Krishzyme Recombinant Enterokinase	500 U
KBENZ46	Krishzyme Recombinant IdeS Protease	1000 U
KBENZ47	Recom Carboxypeptidase B	1 mg
KBENZ48	Recom Endoproteinase Glu-C	1 mg
KBENZ49	Recom Endoproteinase Lys-C	500 ug
KBENZ50	Recombinant Trypsin	100 mg
KBENZ52	Recombinant Trypsin Inhibitor, soybean	100 mg
KBENZ53	Recombinant Human Chymotrypsin C	50 ug
KBENZ71	Krishzyme Recombinant TEV Protease	500 U
KBENZ104	Krishzyme Recombinant Endoproteinase AspN	50 ug
NUCLEASE		
KBENZ61	FastNuclease™ Endonuclease Serratia Marcescens	10 KU
KBENZ62	FastNuclease™ DNase I (RNase-free, Protease-free)	500 KU

As an alternative to Benzonase™ Nuclease, FastNuclease™ is an extracellular Serratia marcescens endonuclease produced from genetically engineered E. coli. It possesses the same activity as Benzonase does, but is much more cost-effective !!!

### FastNuclease™ Activity

FastNuclease™ requires Mg<sup>2+</sup> or Mn<sup>2+</sup> for activity and displays a broad pH range from 6 to 10 (optimal at 8 – 8.5) and a wide temperature optimum between 35°C and 44°C. Maximal activity obtained with 10 mM MgCl<sub>2</sub> was three times that attained with 1 mM MnCl<sub>2</sub>. 1 mM EDTA reduced the activity by 30% in the presence of 1 mM MgCl<sub>2</sub>; 0.1 M EDTA eliminated all enzyme activity.

In the presence of 1 mM MgCl<sub>2</sub>, enzyme levels were reduced 75% by 0.1 M CaCl<sub>2</sub> or 1 M NaCl. Under standard assay conditions, 1 mM iodoacetate had no effect on the enzymatic rate, whereas 1 mM mercaptoethanol and maleic acid reduced the activity by only 5 to 10%. 10 mM p-Chloromercurybenzoate completely inactivates the enzyme, while 0.64 M beta-mercaptoethanol in the presence of 2 M urea causes only partial inactivation of the enzyme. 4 or 7 M Urea increases the enzyme activity.

**KRISHGEN BioSystems**  
OUR REAGENTS, YOUR RESEARCH

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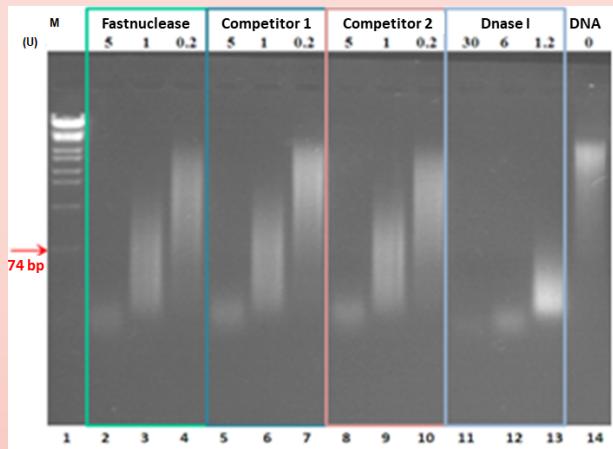
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**discovery**  
THROUGH INNOVATIVE SCIENCE AND TECHNOLOGY

**FASTNUCLEASE™**  
**serratia marcescens**  
**nuclease**

Enzyme & ELISA  
for residual detection



Comparison of the FastNuclease and other nucleases in different amount of nuclease by salmon sperm DNA cleavage assay.

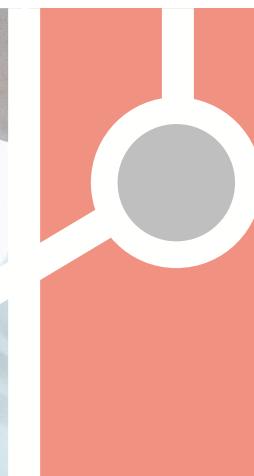
\*The DNase I's activity unit is decided by the FastNuclease's definition.



FastNuclease™ (similar to Benzonase™) is a nonspecific nuclease which cleaves both RNA and DNA in any form (single stranded, double stranded, linear, circular and supercoiled) with similar rates. It hydrolyzes internal phosphodiester bonds present between the nucleotides. All free nucleic acids can be completely digested to 5'-monophosphate-terminated oligonucleotides of 3 to 8 bases in length. Under in vitro conditions, however, FastNuclease has been shown to prefer GC over AT-rich regions, as does DNase I.

The nuclease is a homodimer with monomer molecular masses ranging from 25 to 30 kDa. Two disulfide bonds found in the nuclease are crucial to its activity and stability.

- ◆ FastNuclease™ reduces the viscosity of samples, digest all forms of nucleic acid and enhances protein purification via complete cell lysis and viscosity reduction.
- ◆ It is used to increase gel resolution.
- ◆ FastNuclease™ is also used to prevent cell clumping.



OUR STRATEGY FOR LEADERSHIP IN SCIENCE IS TO PROVIDE THE BEST PRODUCTS AND SOLUTIONS TO ACHIEVE THE BEST RESULTS

## background

Nucleic acids can also be eliminated by enzymatic digestion using FastNuclease™ which -

- Cleaves single and double stranded DNA, RNA
- Reduces or eliminates nucleic acids from the disrupted cells
- Greatly decreases the viscosity of the resultant solution

FASTNUCLEASE™ or *Serratia marcescens* nuclease is a well-known non-specific nuclease. It is widely used in the purification of recombinant proteins because it reduces or eliminates nucleic acids from the disrupted cells, and greatly decreases the viscosity of the resultant solution. This makes subsequent purification procedures easier. Commercial versions of this enzyme are available. The FASTNUCLEASE™ Endonuclease enzyme was compared and the data produced to exhibit its quality to the other manufacturers.

The benefit of working with FASTNUCLEASE™ is that post the cleaving or bio-production process, the residual enzyme is easy to monitor using the FASTNUCLEASE™ Endonuclease ELISA kit.

The key factor is the kit uses antibodies that are specific to the enzyme used in the process earlier ensuring the kit exhibits high specificity. Regulatory bodies like European Medical Agency (EMA) and the US Food & Drug Administration (FDA) have set limits of residual DNA in biological products.

This is due to the fact that residual enzyme in the product may lead to oncogenicity and infectivity of host cell nucleic acid. Manufacturers are constantly working to upgrade the downstream production process to eliminate inherent impurities. The FASTNUCLEASE™ Endonuclease ELISA kit would be an additional support in the monitoring process.

An unrelenting focus on breakthrough science research is the foundation of our success and the heart of KRISHGEN's overall strategy.



### KRIBIOLISA FASTNUCLEASE ELISA Cat No: KBBA36

#### Intended Use:

The Endonuclease *serratia marcescens* ELISA is an enzyme-linked immunosorbent assay for accurate and precise quantitative detection of endonucleases from cell culture supernatant.

#### Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to endonuclease are pre-coated onto microwells. Samples and standards are pipetted into microwells and endonuclease present in the sample are bound by the capture antibody.

Then, a HRP (horseradish peroxidase) conjugated anti-endonuclease is pipetted and incubated simultaneously with samples. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of endonuclease in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Calibrator Range: 0 -10 ng/ml

Sensitivity: 0.2 ng/ml

