Data Sheet

FastNuclease™ Endonuclease *Serratia marcescens*

Cat No: KBENZ61-02

Pack Size: 500 KU

General Information:

Provider: KRISHGEN BIOSYSTEMS

Synonym: Nuclease, *Serratia marcescens'* extracellular endonuclease.

Protein Construction: KRISHZYME™ Endonuclease is a recombined *Serratia marcescens'* extracellular endonuclease.

Source: *Aequorea victoria*

Expression Host: *E.coli*

QC Testing:

Purity: > 99%, as determined by chromatography.

Endotoxin: < 0.22 EU/1000 as determined by the LAL method.

Stability: Samples are stable for up to twelve months from date of receipt -20°C to -80°C.

Predicted N terminal: Three isoforms with different N terminal may be found from the compound - Sm1 (22D-266N), Sm2 (23T-266N) and Sm3 (25E-266N), the activity analysis shows that they were functionally equivalent.

Molecular Mass: The KRISHZYME™ Endonuclease comprises 266 amino acids and has a calculated molecular mass of Sm1 (26708.2 Da), Sm2 (26591.8 Da) and Sm3 (26376.4 Da). The apparent molecular mass of KRISHZYME™ Endonuclease is approximately 26.5 KDa.

Usage Guide:

Shipping: Shipped at Ambient Temperature. The liquid or lyophilized enzyme is stable for at least 21 days when stored at 37ºC (or ambient temperature).

Formulation: KRISHZYME™ Endonuclease is sterile, lyophilized with pH at 8.0 containing -
50 mM Tris-HCl
20 mM NaCl
2 mM MgCl2
5 % trehalose
5 % mannitol, and
0.01 % Triton® (Triton® is a trademark of Dow Chemical, USA). Follow the instructions on the vial.

Centrifuge the vial at 4°C before opening to recover the entire contents. Please contact us for any concerns or special requirements at +91-22-49198700 | Email: sales@krishgen.com

Reconstitution: Follow the instructions on the vial. Centrifuge the vial at 4ºC before opening to recover the entire contents. Normally 25 U/ul is recommended to be the final concentration. For 500KU, add sterile water 2ml, stock solution to 250U/ul.

Storage: Store it under sterile conditions at -20°C to -80°C upon receiving for at least 12 months. Recommend to aliquot the protein into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles.

Protocol:

Large Scale Cell Lysis Treatment:

1. Lysis Buffer Preparation:
   The lysis buffer should be suitable for the protein as well as for downstream purification processes.

2. Re-suspend cell plates in lysis buffer:
   The ratio of lysis buffer (ml) against the gram of cell can be (10-20):1

3. Add KRISHZYME™ Endonuclease (250 units to 1 g cells):
   It is recommended to optimize the amount of KRISHZYME™ Endonuclease.

4. Lysate cell by mechanical or chemical method on ice or at room temperature. Use either an ultrasonic disruptor or high pressure homogenizer or a tissue homogenizer.

5. Obtain clear cell lysate supernatant by centrifugation at ~12,000 rpm for 0.5 hour.

Examples:

1. **Viscosity Reduction of E. coli Lysate:**
   E.coli (1.0 g) with a recombined pET-28a construct was suspended in cell lysate buffer (50 mM Tris-HCl (pH 8.0), 4 M Urea, 100 mM DTT, and 1% Triton X-100), and resulted in 1.0 g/ml. Then cell lysate was incubated with KRISHZYME™ Endonuclease at 4ºC for 5 min. Then samples were centrifuged at 10000 g for 1 min, and photographed (Fig.3).

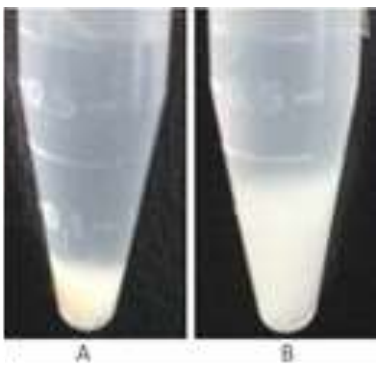

Fig. 3.
A. With 25 U/ml KRISHZYME™ Endonuclease.
B. Without KRISHZYME™ Endonuclease.
Viscosity reduction assay shows that the KRISHZYME™ Endonuclease can reduce the viscosity of E.coli lysate.

2. Salmon Sperm DNA Cleavage Assay:

The substrate Deoxyribonucleic acid sodium salt from salmon testes (Sigma, Catalog # D1626) was diluted with assay buffer (50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 100 µg/ml bovine serum albumin) into 1 mg/ml. Incubate the substrate with different units of KRISHZYME™ Endonuclease as well as other nucleases at 37ºC for 30 min. The DNA fragment was analysed by agarose gel electrophoresis, and photographed (Fig.4).

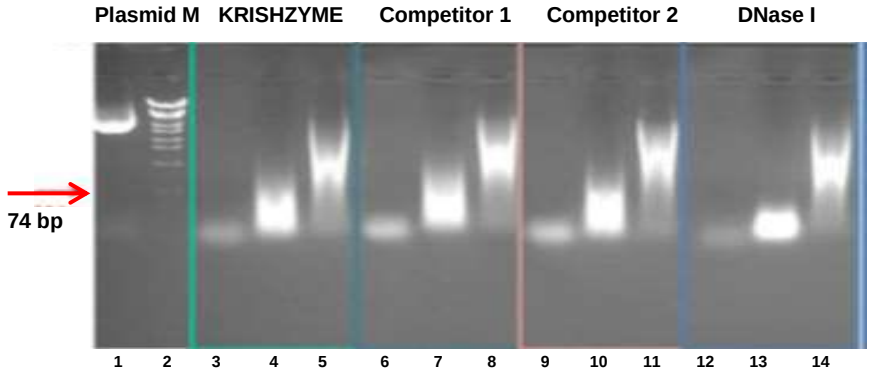

Fig. 5. Comparison of the KRISHZYME™ Endonuclease and other nucleases in different amount of nuclease by plasmid DNA cleavage assay.
*The DNase I's activity unit is decided by the DNase I's definition.

Protein Description:

The KRISHZYME™ Endonuclease is a nonspecific nuclease with high activity, capable of completely digesting RNA and DNA (single stranded, double stranded, linear, circular and super coiled forms, that no fewer than five phosphate residues) into 5'-monophosphate-terminated oligonucleotides of 3-5 bases in length. KRISHZYME™ Endonuclease requires divalent cation, preferably Mg2+ for activity, displays a broad pH tolerance (range from 6 to 10, optimal at 8-8.5) and has a wide temperature optimum between 35ºC and 44ºC. The nuclease is a homodimer (the dimer form is physiologic and functions more progressively than the monomer). Two disulfide bonds in the nuclease are crucial to its activity and stability. It does not have typical protease activity detected by azocasein assay. Its high intrinsic activity and broad substrate tolerance make the endonuclease an ideal tool in a variety of biotechnological and pharmaceutical applications. KRISHZYME™ Endonuclease can be removed by various purification methods.

References:

Friedhoff P, Gimadutdinow O, et al. 1994, Protein Expr Purif, 5 (1): 37-43
Nestle M, Roberts WK. 1969, J Biol Chem, 10, 244 (19): 5213-5218
Michael J. Benedik, Ulrich Strych. 1998, FEMS Microbiology Letters, 165: 1-13 Timothy K.Ball, Yousin Suh.1992, Nucleic Acids Research, Vol. 20, No. 19: 4971-4974
M. Kunitz. 1946, The Journal of Biological Chemistry: 563-568
Christian B. Anfinsen, Robert R. Redfield, et al. 1953, The Journal of Biological Chemistry: 201-210 George N. Eaves, Charles D. Jeffries. 1963, J. Bacteriol. Vol. 85: 273-278
Filimonova M.N., Balaban N. P., Sharipova F. P. 1980, Biokhimiia, 45: 2096-2104 E Schiebel, H Schwarz, and V Braun. J. Biol. Chem., Sep 1989; 264: 16311-16320.
V. Yu. Lunin, V. M. Levdikov, S. V. Shlyapnikov, E. V. Blagova, V. V. Lunin, K. S. Wilson, A. M. Mikhailov. FEBS Letters, Volume 412, Issue 1, 21 July 1997, Pages 217-222.
Pingoud Ingo Franke, Gregor Meiss and Alfred. 1999, J. Biol. Chem. 274:825-832. Filimonova M.N., Krause K.L., Benedik M.J. 1994, Biochem. Mol. Biol. Int. 33:1229-1236

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

The product is For Research Use only and not for Diagnostic or Clinical Use.

Krishgen Biosystems. 2019

THANK YOU FOR USING KRISHGEN PRODUCT!