KANEKA Endonuclease
- Product Information Sheet-

Ver.1.1

KANEKA Endonuclease

1. Introduction

   KANEKA Endonuclease is a genetically engineered endonuclease. It cleaves efficiently all kinds of DNA and RNA into small fragments, irrespective of single-stranded, double-stranded, linear, circular or supercoiled. The enzyme can be used for various applications, such as removal of DNA/RNA from solutions, reduction of cell lysate viscosity caused by nucleic acids, and so on.

2. Product Description

   2.1 Characteristics

   KANEKA Endonuclease is originating from the microorganism *Serratia marcescens*, and expressed in *Pichia pastoris*. A few amino acids substitution is introduced to avoid N-glycosylation, but it did not make any impacts on the original enzyme characteristics. KANEKA Endonuclease is a homodimer of 27 kDa subunit with a calculated pI of pH6.6, and Mg^{2+} is required for enzyme activity.

   2.2 Production

   The host strain *Pichia pastoris* is a yeast species and is free of endotoxins. All the raw materials used for KANEKA Endonuclease manufacturing are free of animal origin and BSE/TSE free. No antibiotics are used in the manufacturing process.

   2.3 Form

   KANEKA Endonuclease is supplied in 50%(v/v) glycerol, 20 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, and 20 mM NaCl.

   2.4 Use

   KANEKA Endonuclease is for research use only. Please feel free to inquire bulk amount and/or GMP grade products.

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3. Enzyme Characteristics

The specifications of KANEKA Endonuclease are summarized in Table 1.

Table 1. Release Specifications of KANEKA Endonuclease

Specification	**Specification Value**
Appearance	Colorless, Clear Transparent
Activity	> 250 U/μl
Specific Activity	> 6.0 x 10^5 U/mg
Purity	≥ 99%
Endotoxin	< 0.25 EU/kU
Protease	Not Detectable
Total Microbial Count	< 10 CFU in 100 kU

3.1 Standard Activity Assay

The following assay method is applied for all activity measurements

Buffer: 50 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 0.1 mg/ml BSA, 1 mg/ml DNA.

Enzyme: KANEKA Endonuclease is diluted to 5-10 mU/μl in 50 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 0.1 mg/ml BSA before use.

- Equilibrate buffer at 37°C
- Start reaction by adding 125 μl of diluted enzyme to 2.5 ml buffer at 37°C. The buffer without DNA is used as control
- The reaction is stopped at 15, 30, 45 and 60 minutes by adding 500μl of the reaction mixture to 500μl of 4% perchloric acid
- The mixtures are incubated on ice for 30 minutes and followed by centrifugation (14,000rpm, 6 minutes, 4°C)
- Measure the supernatant at 260 nm

One unit is defined as the amount of enzyme required to change 260 nm absorbance of 1.0 in 30 minutes. Unit was calculated by a math formula below:

Units/μl = (ΔA260 nm x 30 x V x 2 x F) / (t x v x 1000)

A260 nm = absorbance at 260 nm at time “t”

30 = one unit is defined at 30 min

V =	total assay volume
2 =	dilution factor of the measuring solution
F =	dilution factor of the enzyme solution
t =	incubation time
v =	volume of the enzyme solution
1000 =	conversion factor (ml to µl)

3.2 Specific Activity

The specific activity for KANEKA Endonuclease is measured by the standard assay method as described above. The minimum specific activity for release is 6.0 x 10^5 U/mg.

3.3 Purity

The purity is checked by SDS-PAGE, and we confirmed the dominant band corresponds to KANEKA Endonuclease. The purity of the protein is ≥ 99% and all other protein (< 1%) are derived from host cells, *P. pastoris*.

3.4 Endotoxin

Theoretically, the host strain *P. pastoris* does not contain endotoxin.
The endotoxin level is checked by ENDOSPECY® ES-24S set (SEIKAGAKU CORPORATION), and we confirmed total endotoxin level of all the samples are < 0.25 EU/kU.

3.5 Protease Activity

The protease activity is checked by Colorimetric Protease Assay Kit (Thermo Fisher Scientific), and we confirmed KANEKA Endonuclease is free of detectable protease activity.

3.6 Total Aerobic Microbial Count

The microbial safety is checked by JP Method, and we confirmed as below.
Aerobic bacteria: < 10 CFU in 100 kU , Yeast/Mold: < 10 CFU in 100 kU

3.7 Stability

KANEKA Endonuclease has been stored at different temperatures and the enzyme activity was measured at regular intervals. Based on the results of the stability test and also the results of the accelerated stability test, the shelf

life time of KANEKA Endonuclease is set more than 24 months at -20 °C from the product release date.

3.8 Storage

KANEKA Endonuclease is rather stable, but storage under extreme conditions will lead to activity loss. The optimum storage temperature is -20 °C.

4. Performance

The performances of KANEKA Endonuclease are shown in Table 2 and Figure 1-6. The competitor's product was used for reference in several tests.

Table 2. Condition of Use

Condition	**Recommended**	**Effective**
Mg^{2+}	1 - 2 mM	1 – 20 mM
pH	7.5 - 9.0	6.0 – 10.0
Temperature	30 - 37 °C	0 - 42 °C

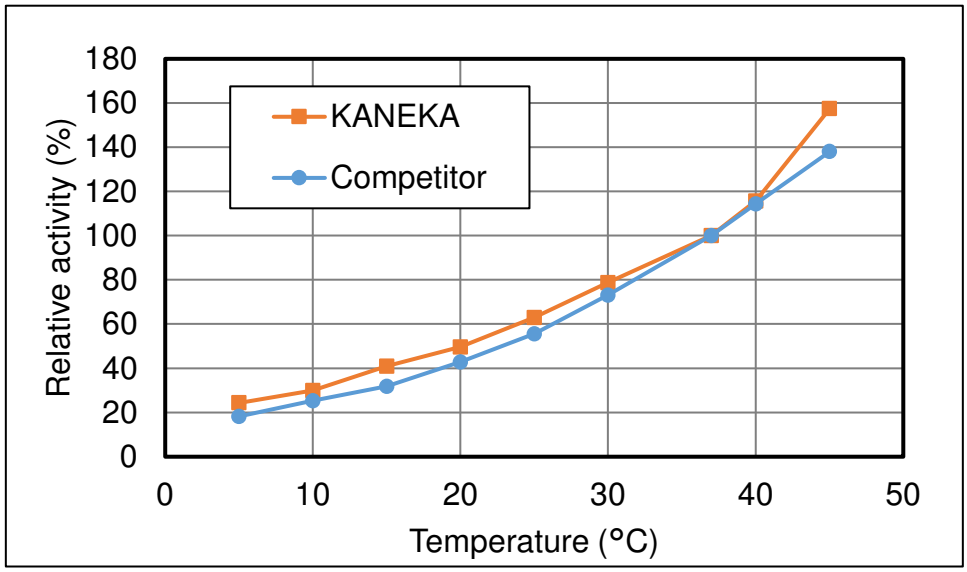

Figure 1: Effect of Temperature

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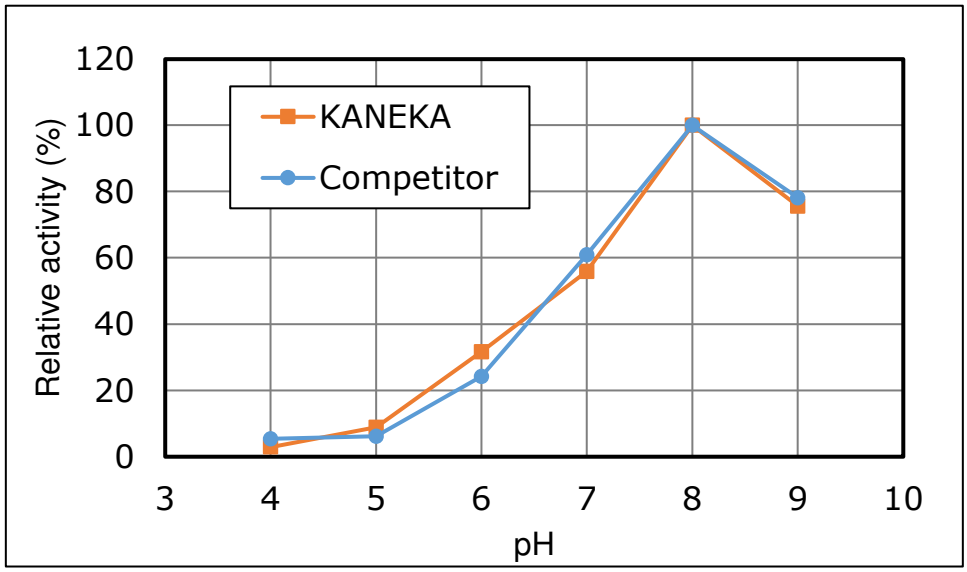

Figure 2: Effect of pH

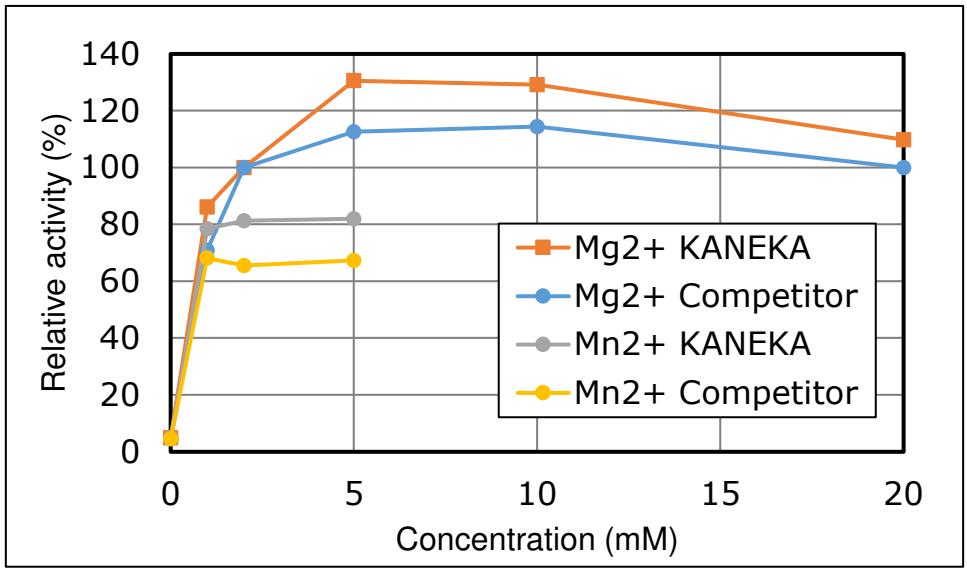

Figure 3: Effect of Magnesium and Manganese Ion Concentration

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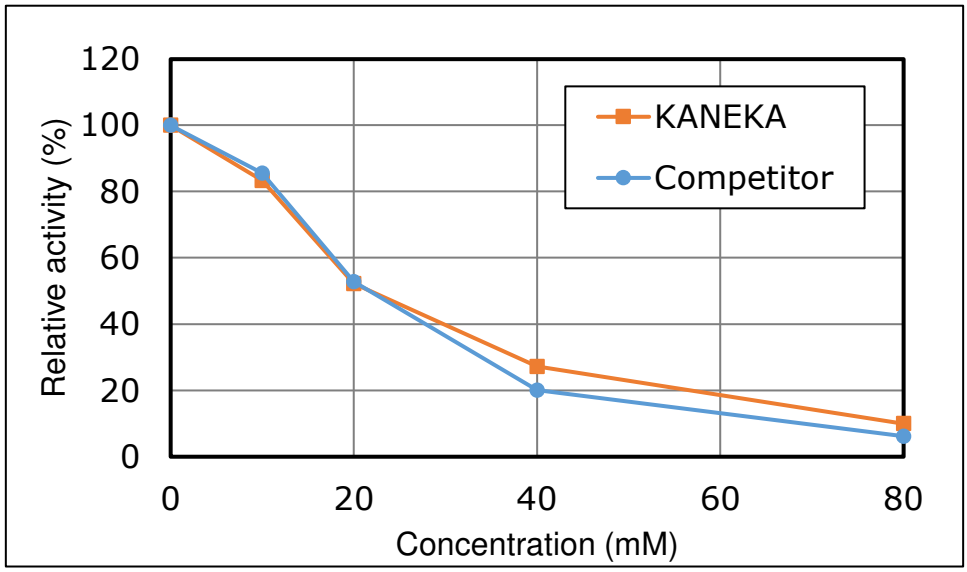

Figure 4: Effect of Phosphate Concentration

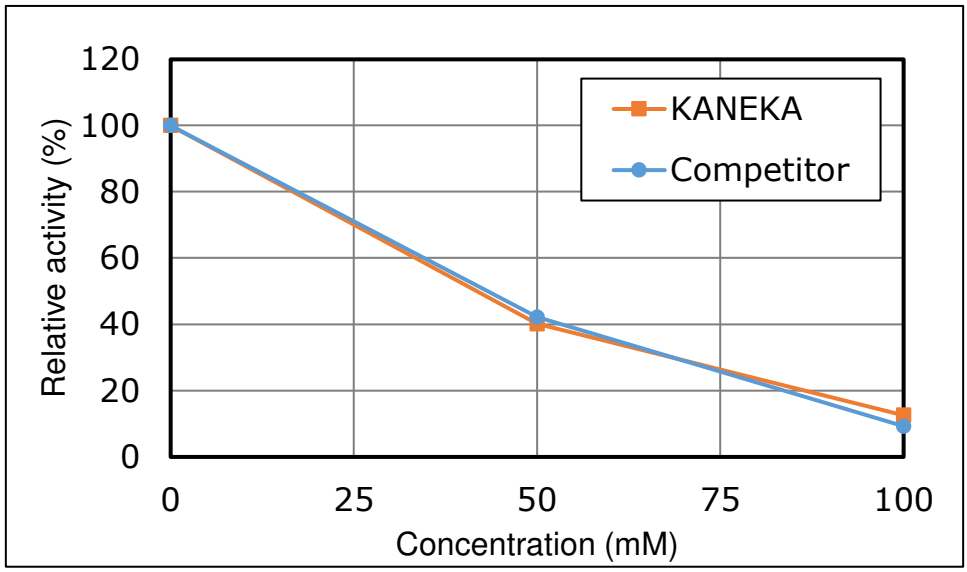

Figure 5: Effect of Ammonium Sulfate Concentration

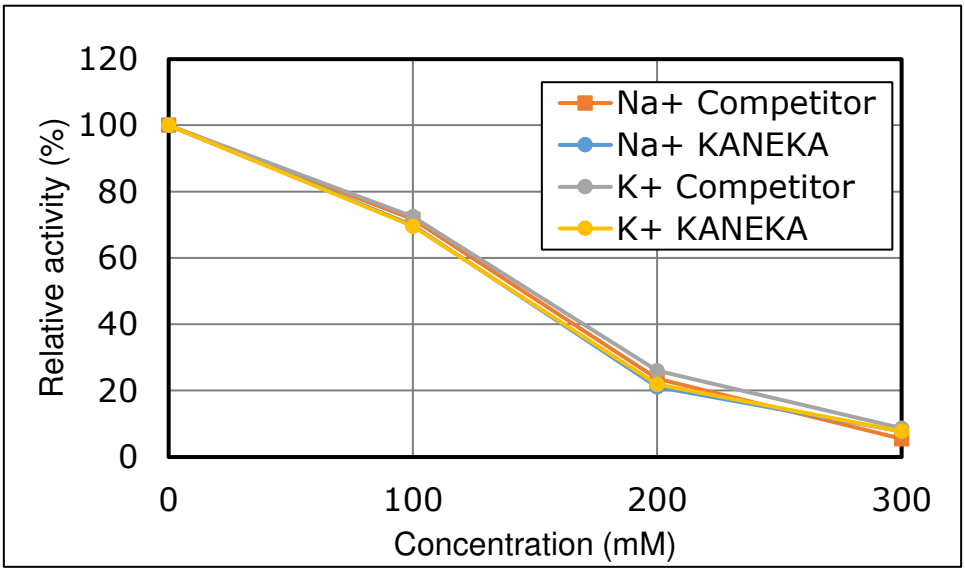

Figure 6: Effect of Monovalent Cation (Na^+, K^+) Concentration

5. Application

KANEKA Endonuclease has high nuclease activity and is generally applied for removal of contaminant DNA and viscosity reduction. Here describes an example of viscosity reduction application.

E. coli cells were suspended in 1L of Tris-HCl buffer pH7.0 to obtain a suspension of OD600 = 100. 10ml of 1M $MgCl_2$ was added. KANEKA Endonuclease was added to obtain a final concentration of 100 U/ml. The cells were lysed on ice with an ultrasonic prove for 30 minutes. The lysate was incubated at 37 ºC for 30 minutes. Following centrifugation at 12,000 x g (15 min., 25 ºC), cell lysate with low viscosity was obtained.

6. Removal of the Enzyme

6.1 Removal

KANEKA Endonuclease can be removed by various methods. One of the highly efficient method is a chromatography during downstream process. By setting appropriate conditions, only the target molecule binds to chromatography resins and KANEKA Endonuclease passes through, or both molecules bind to chromatography resins but elute separately. Alternatively, other methods like ultrafiltration might be applied for removal of KANEKA

Endonuclease. Since the removal efficiency is depending on the conditions, preliminary tests are highly recommended.

6.2 Detection

The amount of KANEKA Endonuclease in sample solutions can be detected and quantified by the Benzonase® ELISA kit II (Merck Millipore). The kit consists of an antibody coated microtiter plate that specifically captures endonucleases from *Serratia marcescens*. After adding horseradish peroxidase conjugated antibodies and TMB, Yellow complex is formed as depending on amount of endonucleases. The reaction is stopped by the addition of 0.2 M H_2SO_4 and the result can be measured by using an ELISA plate reader at 450 nm.

Both *Serratia marcescens* endonucleases showed similar absorbance spectra at 450 nm (Figure 7). This indicates that there is no significant difference in binding affinity between the two endonucleases. Therefore, the Benzonase® ELISA kit II can be used for the quantification of KANEKA Endonuclease.

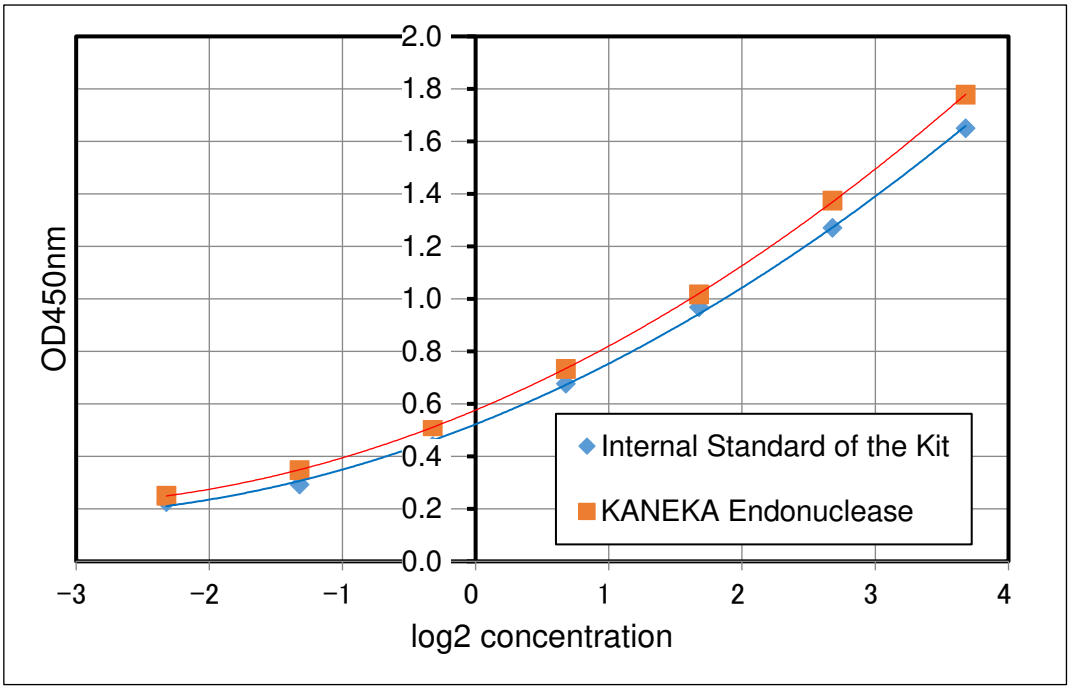

Figure 7: Calculation curve of two endonucleases

7. Sales and Service

7.1 Ordering Information

KANEKA Endonuclease is supplied in two different packaging sizes as below. For bulk amount and/or GMP grade products, please make inquiry.

Product No.	Size	Activity
KEN02100	100 kU	> 250 U/μl
KEN02500	500 kU	> 250 U/μl

7.2 Contact

Kaneka Corporation
Pharma & Supplemental Nutrition Solutions Vehicle
2-3-18, Nakanoshima, Kita-ku, Osaka 530-8288, Japan
E-mail: knkmicpro@kaneka.co.jp
Product web page: https://www.microbial-products-kaneka.com/
Company web page: http://www.kaneka.co.jp/

8. Disclaimers and Warranty

All experimental data are provided “as is” without any warranty of accuracy or completeness.

The range of our responsibility shall be limited to exchange with a replacement product in case there is a defect in this product. We will not be held responsible for any damages caused through use of this product whether they are direct or indirect.