Cat. # 2670A

For Research Use

TaKaRa

Cryonase™ Cold-active Nuclease

Product Manual

v201702Da

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Cryonase Cold-active Nuclease, an endonuclease originated from a psychrophile *Shewanella* sp. and expressed in and purified from recombinant *E. coli*, can digest all types of DNA and RNA substrates (single-stranded, double-stranded, linear, or circularized) at lower temperatures. Exhibiting an activity even on ice, this enzyme is useful for digesting nucleic acids in samples containing proteins or other heat-unstable substances.
This product was developed based on the results of a collaborative study between TaKaRa Bio and Professor Nobuyoshi Ezaki, *et al*. of Kyoto University, Institute for Chemical Research on a genomic analysis of *Shewanella* sp. Ac10 strain supplied by Professor Ezaki's group.

I. Content

Cryonase Cold-active Nuclease 10,000 U (20 U/μl x 500 μl)

II. Form

10 mM	Tris-HCl, pH 7.5
20 mM	NaCl
2 mM	$MgCl_2$
50%	glycerol

III. Source

Expressed in recombinant *E. coli*

IV. Storage

-20℃

V. Unit Definition

One unit of the enzyme is defined as the amount that increases the absorbance at 260 nm of the reaction mixture (3 ml) by 0.001 in a minute at 37℃ and pH 7.5, with salmon sperm DNA as a substrate.

VI. Composition of Reaction Mixture for Unit Definition

83 mM	Tris-HCl, pH 7.5
4.2 mM	$MgCl_2$
33 μg/ml	Substrate DNA

VII. Purity

The presence of protease contamination is less than the detection limit.

VIII. Usage

- Degradation of genomic DNA
- Reducing viscosity when extracting proteins from bacterial cells
- Pretreatment of samples for two-dimensional electrophoresis
- Pretreatment for virus purifications

IX. Examples

Example 1: Reducing viscosity when extracting proteins from *E. coli*

Following experiment assessed the reduction in viscosity when Cryonase Cold-active Nuclease was added in place of DNase I in extracting proteins using TALON® xTractor™ Buffer Kit (Cat. #635623: The product name changed to xTractor Buffer Kit at present, and there are a few changes in the specification of the product.).

[Method]

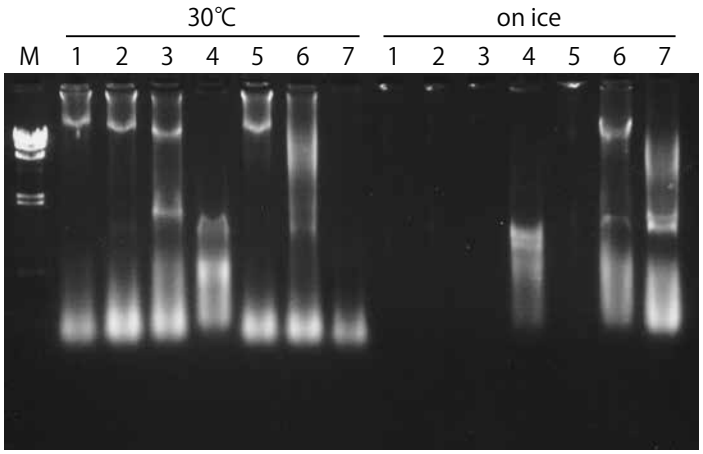
This experiment was performed in accordance with the protocol for TALON xTractor Buffer Kit.

Aliquots of 6.4 mg wet mass of *E. coli* BL21 expressing protein 'A' were added with 128 μl of the TALON xTractor Buffer, 1.28 μl Lysozyme (50X), and either DNase I or Cryonase Cold-active Nuclease each in one of three unit sizes (0.256 U, 2.56 U, and 25.6 U) and left at 30℃ or on ice for 30 minutes to allow for reaction.

[Results]

(1) *E. coli* genomic DNA analysis by agarose gel electrophoresis

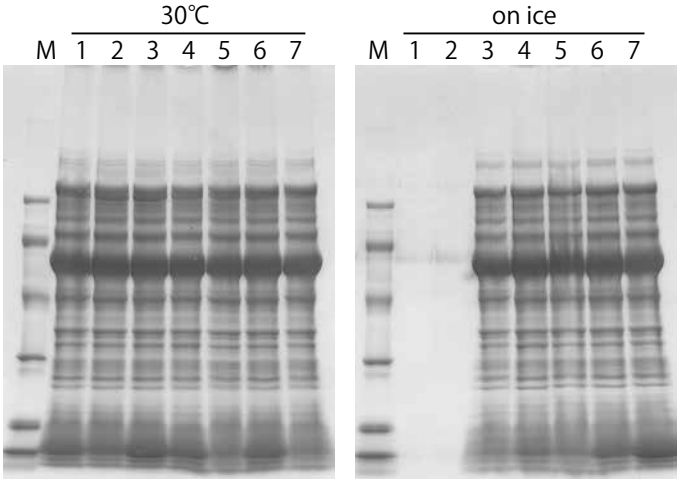
A 10 μl aliquot of each extraction mixture was used for electrophoresis.



M : λ-*Hin*d III digest
1 : without nuclease
2 : +DNase I, 0.256 U
3 : +DNase I, 2.56 U
4 : +DNase I, 25.6 U
5 : +Cryonase Cold-active Nuclease, 0.256 U
6 : +Cryonase Cold-active Nuclease, 2.56 U
7 : +Cryonase Cold-active Nuclease, 25.6 U
(The protocol for xTractor Buffer Kit specifies the use of 0.256 U.)

On the above electrophoresis profile, some lanes showing no bands because of inappropriate application of samples due to the viscosity in pipetting.
The experiment demonstrated that with treatment on ice, DNase I, even at 2.56 U, did not lower the viscosity enough to resolve the difficulty in pipetting, but Cryonase Cold-active Nuclease at 2.56 U decreased the viscosity by genomic DNA degradation.

(2) Analysis of extracted proteins by SDS-PAGE
The extraction mixtures were mixed with a sample buffer and boiled. Then a 4 μl-equivalent of each extraction mixture was applied to SDS-PAGE.



M : Marker
(97, 66, 45, 31, 21, 14 kDa)
1 : without nuclease
2 : +DNase I, 0.256 U
3 : +DNase I, 2.56 U
4 : +DNase I, 25.6 U
5 : +Cryonase Cold-active Nuclease, 0.256 U
6 : +Cryonase Cold-active Nuclease, 2.56 U
7 : +Cryonase Cold-active Nuclease, 25.6 U
(The protocol for xTractor Buffer Kit specifies the use of 0.256 U.)

With treatment on ice, Cryonase Cold-active Nuclease exhibited the same viscosity-lowering effect as DNase I at a unit size of one order smaller.
With its ability to lower viscosity on ice without affecting proteins, this enzyme can be useful in protein extractions.

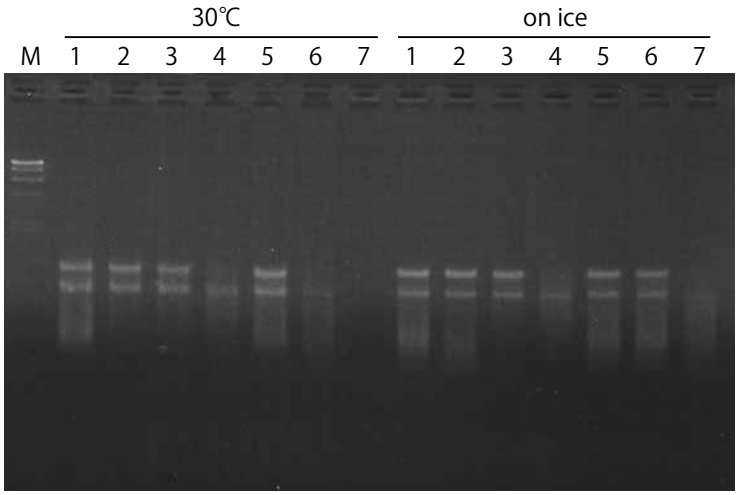
Example 2: Degradation of genomic DNA in *E. coli* supernatant by ultrasonication

E. coli supernatant samples were mixed with either Cryonase Cold-active Nuclease or DNase I, and the amounts of residual *E. coli* genomic DNA were compared.

[Method]
E. coli BL21 expressing protein 'A' was disrupted by ultrasonication in 5 ml buffer/g wet volume (buffer: 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$), and the supernatant was diluted by 100-fold using the same buffer. Aliquots of 50 μl of the diluted supernatant were added with either DNase I or Cryonase Cold-active Nuclease each in one of three amounts (0.1 U, 1 U, or 10 U) and kept at 30℃ or on ice to allow for reaction. Samples were collected 30 minutes and 2 hours later for analysis by agarose gel electrophoresis. A 10 μl aliquot of each reaction mixture was used for electrophoresis.

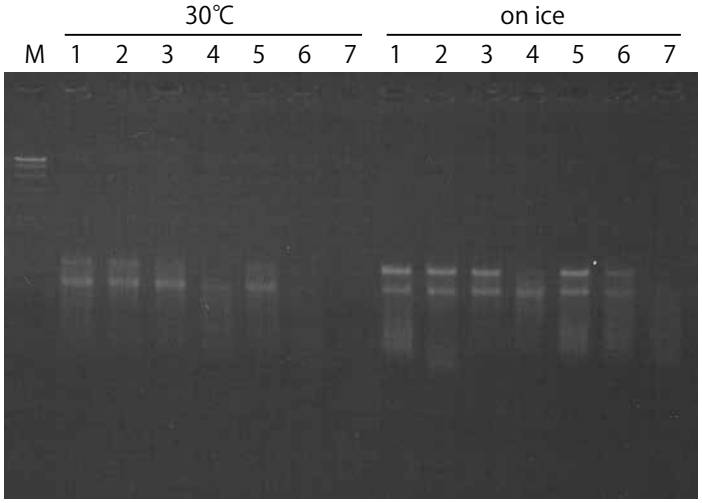
[Results]

(1) 30 minutes



M : λ -*Hin*d III digest
1 : without nuclease
2 : +DNase I, 0.1 U
3 : +DNase I, 1 U
4 : +DNase I, 10 U
5 : +Cryonase Cold-active Nuclease, 0.1 U
6 : +Cryonase Cold-active Nuclease, 1 U
7 : +Cryonase Cold-active Nuclease, 10 U

(2) 2 hours

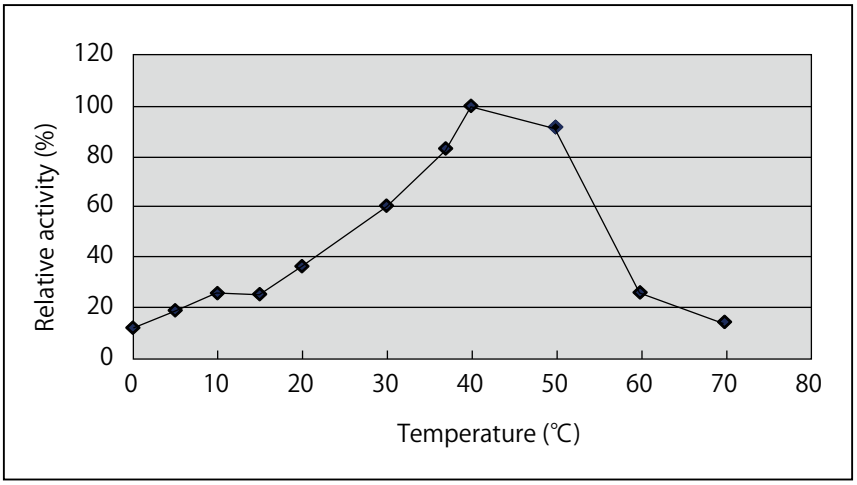


M : λ -*Hin*d III digest
1 : without nuclease
2 : +DNase I, 0.1 U
3 : +DNase I, 1 U
4 : +DNase I, 10 U
5 : +Cryonase Cold-active Nuclease, 0.1 U
6 : +Cryonase Cold-active Nuclease, 1 U
7 : +Cryonase Cold-active Nuclease, 10 U

The results demonstrated that Cryonase Cold-active Nuclease provided excellent genomic DNA degradation even on ice. With its ability to degrade DNA without affecting the target protein in low-temperature reactions, this enzyme is useful in protein extractions and purifications.

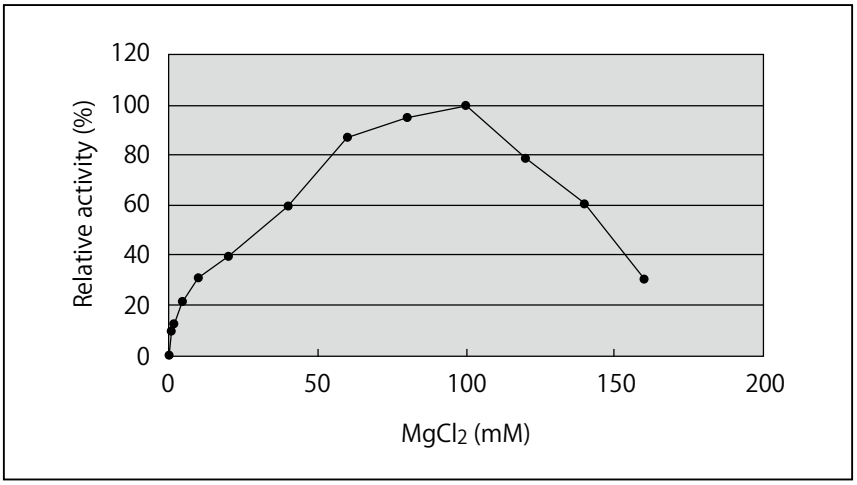
X. Specifications

■ Relative activity at different temperatures



■ Effect of pH
Range of optimum pH : 7.0 - 9.0
Range of effective pH : 6.0 - 10.0

■ Relative activity at various Mg^{2+} concentrations (pH 7.5, 37℃)
Range of optimum concentration : 60 - 120 mM



XI. Related Products

xTractor™ Buffer Kit (Cat. #635623)
Recombinant DNase I (RNase-free) (Cat. #2270A)
pCold™ Vector Set (Cat. #3360)*
pCold™ I DNA (Cat. #3361)*
pCold™ II DNA (Cat. #3362)
pCold™ III DNA (Cat. #3363)
pCold™ IV DNA (Cat. #3364)
pCold™ TF DNA (Cat. #3365)*
pCold™ ProS2 DNA (Cat. #3371)*
pCold™ GST DNA (Cat. #3372)*
SPP System™ I (Cat. #3367)*
SPP System™ II (Cat. #3368)
SPP System™ III (Cat. #3369)
SPP System™ IV (Cat. #3370)
SPP System™ Set (Cat. #3366)*

[*E. coli* Competent Cells]
Chaperone Competent Cells BL21 Series (Cat. #9120 - 9125)

[His-Tag Fusion Protein Purification Reagents]
Capturem™ His-Tagged Purification Miniprep Kit (Cat. #635710)
Capturem™ His-Tagged Purification Maxiprep Kit (Cat. #635713)
TALON® Metal Affinity Resin (Cat. #635501)
HisTALON™ Gravity Column Purification Kit (Cat. #635654)
HisTALON™ Superflow Cartridge Purification Kit (Cat. #635649)
HisTALON™ Buffer Set (Cat. #635651)
His60 Ni Superflow Resin (Cat. #635659)
His60 Ni Gravity Columns (Cat. #635657)
His60 Ni Buffer Set (Cat. #635665)

* Not available in all geographic locations. Please check for availability in your area.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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