FUJIFILM Wako

Code No. 137-14731 (1 vial)

Micrococcal Nuclease
ミクロコッカスヌクレアーゼ

Micrococcal Nuclease catalyzes cleavage of both RNA and DNA to yield 3'-nucleotides[1]. It exhibits exo- and endo-5'-phosphodiesterase activities. It is more specific for single-stranded nucleic acids, and catalyzes preferential cleavage of RNA and DNA at AU or AT-rich regions[2),3)]. The enzyme requires Ca^{2+} for activation[4)]. The reactions can be stopped by EDTA or EGTA.

Source : *Staphylococcus aureus*

Appearance : lyophilized powder

Activity : displayed on label

Molecular Weight : 16,800[5)]

[Method of enzyme activity]

1. Reagents

A. 0.1 mol/L Sodium Borate Solution, pH 8.8
Prepare 180 mL in deionized water using 1.24 g of Boric Acid and adjusting to pH 8.8 with 1 mol/L NaOH and add water to bring the volume up to 200 mL.

B. 0.01 mol/L Calcium Chloride Solution
Prepare 100 mL in deionized water using 0.111g of Calcium Chloride.

C. 2.5 mg/mL Deoxyribonucleic Acid Solution, from Calf Thymus
Dissolve 25 mg of deoxyribonucleic acid, from calf thymus in 10 mL of 0.01 mol/L NaCl. Allow to stand overnight at room temperature and then stir slowly.

D. Enzyme Solution (1,000-fold, 2,000-fold, 4,000-fold, 8,000-fold dilution)
First, dissolve 1 vial of micrococcal nuclease in 1 mL of the below Solution F to make Solution I.
Prepare 10 mL in Solution F using 0.1 mL of Solution I to make Solution II.
Then prepare 10 mL in Solution F using 1 mL of Solution II (1,000-fold dilution).
Add 0.5 mL of Solution F to 0.5 mL of 1,000-fold dilution (2,000-fold dilution).

Add 0.5 mL of Solution F to 0.5 mL of 2,000-fold dilution (4,000-fold dilution).
Add 0.5 mL of Solution F to 0.5 mL of 4,000-fold dilution (8,000-fold dilution).

E. 7% Perchloric Acid
Prepare 10 mL in deionized water using 1 mL of Perchloric Acid (70 %).

F. 0.1 % Bovine Serum Albumin
Prepare 200 mL in deionized water using 0.2 g of Bovine Serum Albumin.

2. Procedure

Reagent	Test	Blank
Solution A	0.1 mL	0.1 mL
Solution B	0.05 mL	0.05 mL
Solution C	0.1 mL	0.1 mL
Incubate at 37℃ for 6～7 min.		
Solution D	0.1 mL (each diluted solution)	–
Solution F	–	0.1 mL
Incubate at 37℃ for 30 min.		
Solution E	0.5 mL	0.5 mL
Chill on ice for 10 min.		
Deionized water	2.7 mL	2.7 mL
Centrifuge for 15 min.		
Determine the A_{260}nm of the supernatant, deionized water as a reference.		

3. Unit Definition

One unit corresponds to a change in optical density of 1.0 at 260 nm at 37℃, using DNA as the substrate.

(Calculation)

$$A = \frac{(E_1 - E_2) \times b}{0.1}$$

A = activity (units/vial)
E_1 = Absorbance of Test sample
E_2 = Absorbance of Blank sample
b = dilution ratio

[Storage] 2 – 10℃ in the dark

[Packages] 1 vial

[References]

1) Alexander, M., Heppel, L. and Hurwitz, J. : *J. Biol. Chem*., **236**, 3014 (1961).
2) Roberts, W., Dekker, C., Rushisky, G. and Knight, C. : *Biochem. Biophys. Acta*., **55**, 664 (1962).
3) Rushinsky, G., Knight, C., Roberts, W. and Dekker, C. : *Biochem. Biophys. Acta*., **55**, 674 (1962).
4) Heins, J. N., Suriano, J. R., Taniuchi, H. and Anfinsen, C. B. : *J. Biol. Chem*., **242**, 1016 (1967).
5) Taniuchi, H., Anfinsen, C. B. and Sodja, A. : *J. Biol. Chem*., **242**, 4752 (1967).

FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome, Chuo-Ku, Osaka 540-8605, Japan
Telephone : + 81-6-6203-3741
Facsimile : + 81-6-6201-5964
http://ffwk.fujifilm.co.jp

FUJIFILM Wako Chemicals U.S.A. Corporation
1600 Bellwood Road
Richmond, VA 23237
U.S.A.
Telephone : + 1-804-271-7677
Facsimile : + 1-804-271-7791
http://www.wakousa.com

FUJIFILM Wako Chemicals Europe GmbH
Fuggerstrasse 12
D-41468 Neuss
Germany
Telephone : + 49-2131-311-0
Facsimile : + 49-2131-311100
http://www.wako-chemicals.de

2306KA2