Biomatik
Tel: (519) 489-7195, (800) 836-8089
Fax: (519) 231-0140, (877) 221-3515
Email: info@biomatik.com
http://www.biomatik.com

Product Information

Version 3.1.01

A2442 DNase I (Deoxyribonuclease I)

Source:	from Bovine Pancreas
Activity:	>500 Kunitz units/mg
CAS #:	9003-98-9
MW:	31 kDa
Storage:	Store at -20°C.

Description:
Bovine pancreatic deoxyribonuclease I (DNase I) is an endonuclease which splits phosphodiester linkages, preferentially adjacent to a pyrimidine nucleotide yielding polynucleotides with free hydroxyl group at the 3' position and phosphate group at the 5' position.

DNase I is activated by bivalent metal ions (Ca^{2+}, Mg^{2+}), and inhibited by chelating agents such EDTA and sodium dodecyl sulfate. DNase I can be stabilized against proteolytic digestion by 5mM Ca^{2+}. Optimal pH: 7-8.

Unit Definition:
One Kunitz unit will produce A260 of 0.001 per minute at pH 5.0 at 25°C using DNA, type I or III as the substrate.

Reconstitution:
Reconstitute the lyophylizate in sterile double-distilled water (10 mg/ml); further dilution with PBS (phosphate buffered saline), HBSS (Hank′s Balanced Salt Solution) or medium.

Preparation Note:

1. *DNase I is sensitive to physical denaturation. Mix gently by inverting the tube. Do not vortex.*

2. For each cell type the working concentration has to be determined individually. For optimal activity the enzyme needs 5 mM Mg^{2+}.

3. Under different buffer conditions the amount of DNase required to completely digest a given amount of DNA may need to be empirically determined. For example, salt concentration >100mM will reduce DNase activity.

4. DNase I is sensitive to physical denaturation. Mix gently by inverting the tube. Do not vortex.
   DNase I solution at 10 mg/ml in 150 mM NaCl may lose 10% of its activity when stored for a week at -20°C.
   DNase I solution at 10 mg/ml in 150 mM NaCl may lose 20% of its activity when stored for a week at 4°C.

Enzyme Activity

Enzyme activity is assayed in the following procedure:

1) Reagent preparation

a) 1M sodium acetate buffer with PH=5.0: Weigh 4.1g anhydrous sodium acetate, add 40ml pure water to dissolve, adjust pH=5.0 with concentrated hydrochloric acid, and finally make up to 50ml.
b) 100 mM magnesium sulfate solution: Weigh 1.23 g of magnesium sulfate heptahydrate, dissolve it in pure water and dilute to 50 ml.
c) 0.85% NaCl solution: Weigh 0.425g of sodium chloride, add pure water to dissolve and dilute to 50ml.
d) DNA solution: Weigh 3.3 mg of sodium deoxyribonate type I. Add 10 ml of pure water to dissolve on ice and dissolve for at least 30 min or more until completely dissolved.
e) Enzyme solution of DNase I (>500U/mg). Weigh 4.28mg, add 5ml 0.85% NaCl solution to dissolve.
f) Substrate solution: 5 ml of pH=5 buffer solution, 2.5 ml of 100 ml of magnesium sulfate solution, 6 ml of DNA solution, and finally made up to 50 ml with pure water in a 50 ml volumetric flask.
g) Blank reference solution: 5 ml of pH=5 buffer solution, 2.5 ml of 100 ml of magnesium sulfate solution, and finally made up to 50 ml with pure water in a 50 ml volumetric flask.

2) Experimental steps

Activity assay	TEST
Substrate solution	2.9
Enzyme solution	0.1

The reading is recorded every 1 minute at 260 nm, the change value of the absorbance per minute is calculated, and the maximum change value is taken to calculate the activity.

3) Calculation formula

$CF= R_{std} / T_{std}$
Activity= K * 3 * 5 / 0.001 * v *m
Corrected activity = activity x CF
Where: CF- activity correction factor
R_{std} - standard nominal activity, U/mg
T_{std} - standard test activity, U/mg
V - the volume of the enzyme solution added, ml
3- Total volume of reaction solution, ml
5-Preparation of the volume of the enzyme solution, ml
0.001-A260nm per 0.001 absorbance is defined as one unit activity

This material is for laboratory research purpose and/or in vitro use only and is not to be used in humans or animals.