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# ProductInformation

**AGARASE I (EC 3.2.1.81)**
Product Number **A 8688**
Lot Number 072K0729
Storage Temperature –20 °C

## Product Description

SOURCE: *Psuedomonas atlantica*
Activity: 1 unit/ µl
DNase: None detected
RNase: None detected
Nickase: None detected
Protease: None detected
Functional Test: Suitable for use in isolating DNA fragments from agarose gels.

## Introduction

Agarase I is commonly used for isolating of DNA fragments of all sizes from agarose gels[1-4] prepared with low melting point agarose. This method[3] is especially gentle and therefore useful for the isolation of very large DNA fragments (up to 680 kb) suitable for cloning pYAC libraries. This enzyme degrades melted agarose to oligosaccharides[5] that will not gel on cooling. Agarase I and the oligosaccharides often do not interfere with subsequent manipulations, restriction endonuclease digestions, ligations or transformations for example. If necessary, the DNA can be separated from the agarase I reactants by routine phenol extraction and ethanol precipitation.[2]

## Storage Buffer

40 mM Tris-HCl, pH 7.5
50 mM NaCl
50% glycerol

## Assay Buffer

30 mM Bis-Tris, pH 7.1 at 25 °C
10 mM EDTA

## Unit Definition

One unit is the amount of enzyme that digests 100 µl of melted 1% low-melting agarose to neoagaro-oligosaccharides in 1 hour at 45 °C.

## Usage

Cut the band of interest from the low melting point agarose (melting point 65 °C) gel. Place it in a tared reaction tube and determine the weight of the agarose slice (100 mg = 100 µl agarose gel). Add 0.04 volumes of 25X agarase I buffer (0.75 M Bis-Tris, 0.25 M EDTA, pH 7.1 at 25 °C). Incubate at 65 °C for 15 min. or until the agarose is melted. Cool the agarose to 45 °C. Add 1-2 units (1 unit for gels in 1X TAE, 2 units for gels in 1X TBE) agarase I per 100 mg of 1% agarose gel. Incubate for 1 hr at 45 °C. Note:Increase the agarase I proportionally for higher percentage agarose gels. Smaller DNA fragments (< 50 kb) can be removed from the reactants by ethanol precipitation as follows:

1. Add 0.1 volume 3 M sodium acetate pH 5.2 (Product No. S 7899) to the melted agarose solution and incubate 15 minutes on ice.
2. Centrifuge 15 minutes to pellet the oligosaccharides.
3. Remove the supernatant and place it in a suitable centrifuge tube and add 3 volumes of ice cold ethanol.
4. Centrifuge at 14,000 x *g* for 25 minutes and aspirate the ethanol.
5. Air dry the DNA pellet and dissolve in DNase free water (Product No. W 4502), 1X TE (Product No. T 9285 diluted 1:100) or any other suitable buffer.

## Functional Test

λDNA Hind III digest was run in a 1% agarose gel prepared from low melting agarose (Product No. A 9414) in 1X TBE (Product No. T 6400) running buffer. 6 bands (2.0-2.3kb) were excised and isolated as described in USAGE above. The isolated fragments were not degraded.

**Activity Assay**
Agarase is added to a reaction mixture containing assay buffer and melted agarose in 1 X TAE. The mixture is incubated 1 hour at 45 °C. The reaction is stopped by placing the reaction in an ice bath. After the tubes have cooled to 0 °C the reactions are tested for pipetablility. The least amount of enzyme that resulted in a pipetable reaction is defined as one unit.

**DNase, RNase and Nickase**
Nuclease assays are performed in assay buffer containing 5 units of agarase I and either λ-Hind III digest DNA, tRNA or pBR322 DNA and incubated 16 hours at 45 °C. The results were analyzed by agarose or polyacrylamide gel electrophoresis. No detectable DNase, RNase or nickase was found. Detection limit for DNase and RNase is > 10% degradation and for nickase is 1% conversion from supercoiled to linear or nicked circular DNA.

**Protease**
0.5% FITC-Casein was incubated with 10 µl of enzyme in a 50 µl reaction mixture for 1 hour at 37 °C. Liberated FITC equivalents are quantitated fluorometrically.

Detection limit: 8.5 X $10^{-6}$ µmoles of FITC released per minute.

**References**

1. Burmeister M. F., and Lehrach H., Science **236**, 1305 (1987).
2. Burmeister M., and Lehrach, H., Trends in Genetics, **5**, No 2, 41 (1989).
3. Imai, T. and Olson, M., Genomics, **8**, 297 (1990).
4. Stefano, V., et al., Biochemistry International, **27**, 45 (1992).
5. Yaphe, W., Can. J. Microbiol., **3**, 987 (1957).


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