

CERTIFICATE OF ANALYSIS

# Agarase

#E00461 100u

Lot: Quality guaranteed:

Concentration: 0.5u/ $\mu$ l

Store at  $-20^{\circ}\text{C}$

2  
In total 1 vial.

## Description

Agarase specifically digests the agarose polysaccharide core made up of repeating 1,3-linked  $\beta$ -D-galactopyranose and 1,4-linked 3,6-anhydro- $\alpha$ -L-galactopyranose into neo-agaro-oligosaccharides (1).

## Source

*E.coli* cells carrying a plasmid with a cloned gene encoding  $\beta$ -agarase from *Pseudomonas atlantica*.

## Unit Definition

One unit of the enzyme completely degrades 100 $\mu$ l (approx. 100mg) of molten 1% low melting point agarose in 30min at  $42^{\circ}\text{C}$ .

## Activity Assay Buffer (1X TBE Buffer)

89mM Tris base, 89mM boric acid, 2mM EDTA.

## Storage Buffer

50mM Tris-HCl (pH 7.5), 0.1M NaCl, 0.1% Triton X-100 and 50% glycerol.

## Applications

Quantitative recovery of DNA or RNA from low melting point agarose gels (see *protocol on back page*). The recovered nucleic acids can be used directly for sequencing, amplification, etc.

## Inactivation

By heating at  $70^{\circ}\text{C}$  for 10min.

## Note

- Activity of Agarase in different buffers (in comparison to activity in assay buffer):

TBE (90mM Tris-borate, 2mM EDTA, pH 8.3)	100%
TAE (40mM Tris-acetate, 1mM EDTA, pH 8.5)	120%
TPE (90mM Tris-phosphate, 2mM EDTA, pH 7.7)	120%
Bis-Tris (50mM Bis-Tris-HCl, 1mM EDTA, pH 6.5)	150%
- Ethidium bromide up to 5µg/ml does not inhibit Agarase.
- Agarase has a molecular weight of 32.7kDa.
- The conventional agarose is not suitable for digestion by Agarase. However, the MetaPhor<sup>®</sup> intermediate melting temperature agarose by FMC may be digested successfully using Agarase.

## QUALITY CONTROL ASSAY DATA

### **Endodeoxyribonuclease Assay**

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 5 units of Agarase with 1µg of pBR322 DNA in 50µl of buffer (33mM Tris-HCl (pH 7.9 at 37°C), 10mM magnesium acetate, 66mM potassium acetate) for 4 hours at 37°C.

### **Exodeoxyribonuclease Assay**

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 5 units of Agarase with 1µg of sonicated *E.coli* [<sup>3</sup>H]-DNA in 50µl of buffer (33mM Tris-HCl (pH 7.9 at 37°C), 10mM magnesium acetate, 66mM potassium acetate) for 4 hours at 37°C.

## **Ribonuclease Assay**

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 5 units of Agarase with 1µg of [<sup>3</sup>H]-RNA in 50µl of buffer (33mM Tris-HCl (pH 7.9 at 37°C), 10mM magnesium acetate, 66mM potassium acetate) for 4 hours at 37°C.

### **Labeled Oligonucleotide (LO) Assay**

No detectable degradation of a single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 5 units of Agarase for 4 hours at 37°C.

### **DNA recovery**

>90% recovery of 2µg of a DNA ladder ranging from 0.25kb to 10kb in length.

### **Blue/White Cloning Assay**

The mix of pUC57 DNA/HindIII, pUC57 DNA /PstI and pUC57 DNA /Eco32I digests was recovered from low melting point agarose using Agarase. The DNA was then ligated. 0.2% of white colonies were detected after transformation of competent *E.coli* XL1-Blue cells with ligated DNA.

Quality authorized by:

 Jurgita Zilinskiene

(continued on back page)

# ***Protocol for the Recovery of DNA from Low Melting Point Agarose Gels***

## **I. AGAROSE DIGESTION**

- ➊ Perform electrophoresis of DNA in a low melting point agarose gel prepared in TAE, 0.5XTBE, TBE or TPE buffer. Stain the gel with ethidium bromide.
- ➋ Cut out the desired band from the agarose gel with a clean, nuclease free spatula. Limit UV exposure of the gel slice to a minimum. Cut out only as much agarose as is necessary to recover the DNA band.
- ➌ Place the gel slice into a pre-weighed 1.5ml microcentrifuge tube and determine the weight of the slice. To facilitate melting, cut gel slices larger than 200mg into smaller pieces.
- ➍ Incubate the tube for approx. 10min at 70°C until the agarose is **completely** melted.

### ***Note***

- Incubation at elevated temperatures may denature DNA.
  - Ensure that the gel slice is thoroughly melted. If the agarose is not completely melted, hydrolysis also will be incomplete.
- ➎ Transfer the tube to a 42°C water bath and equilibrate for 5min prior to adding Agarase.
  - ➏ Add 1 unit of Agarase per 100mg (approx. 100µl) of 1% agarose; gently mix and incubate for 30min at 42°C.

### ***Note***

- If you are using a higher percentage agarose, the amount of Agarase should be proportionately increased.

## **II. DNA PURIFICATION**

### *DNA fragments larger than 30 kb*

Large DNA fragments require delicate handling to avoid mechanical shearing.

- ➊ Centrifuge at 15000xg for 10min to pellet undigested carbohydrates.
- ➋ Remove oligosaccharides and Agarase by dialysis or carry out subsequent manipulations with DNA in the digested agarose solution.

### *DNA fragments smaller than 30 kb*

- ➌ Add salt to hydrolyzed agarose: ammonium acetate to 2.5M or sodium acetate to 0.3M.

### ***Note***

- Use ammonium acetate rather than other salts, because they may cause co-precipitation of oligosaccharides with DNA.
  - T4 Polynucleotide Kinase is inhibited by ammonium ions. Use sodium acetate if, following recovery, you will be labeling 5'-ends of DNA with T4 Polynucleotide Kinase.
- ➍ Chill on ice for 5min, centrifuge at 15000xg for 10min to pellet undigested carbohydrates.
  - ➎ Transfer the supernatant to a clean tube. Add 1 volume of isopropanol or 2-3 volumes of ethanol, mix gently and incubate for at least 30min at 0°C to 22°C.

### ***Note***

- If DNA fragments are <500 bp or if DNA concentration is <0.05µg/ml, incubate overnight at 0°C to 22°C.

- ④ Centrifuge at 15000xg for 15min, remove supernatant and dry pellet. The pellet can be resuspended in an appropriate buffer for subsequent manipulation.

### **Reference**

1. Yaphe, W., The use of agarase from *Pseudomonas atlantica* in the identification of agar in marine algae (*Rhodophyceae*), Can. J. Microbiol., 3, 987-993, 1957.

### **Related Products**

- Top Vision™ LM GQ Agarose #R0801
- Water, nuclease-free #R0581  
#R0582

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### **PRODUCT USE LIMITATION.**

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Please refer to [www.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.