

Product: Agarase

CATALOG #: 9201-500

AMOUNT: 500 units

LOT #: _____

SOURCE: E. coli

DESCRIPTION:

The agarase is a recombinant protein from *P. atlantica* produced in *E. coli*. Agarase cleaves agarose to neoagarose-oligosaccharides, and is used for quantitative, gentle recovery of DNA from low melting point agarose.

CONCENTRATION: 2 units/ μ l

UNIT DEFINITION:

One unit is defined as the amount of enzyme required to digest 100 μ l (approx. 100 mg) of molten 1% low melting point agarose to neoagarose-oligosaccharides in 1 hour at 45°C.

ENZYME STORAGE BUFFER: 40 mM Tris-HCl, pH 7.5, 50 mM NaCl, 50% glycerol (v/v).

STORAGE CONDITIONS: -20°C

10X BUFFER (Supplied): 1 M Tris-HCl (pH 6.5), 10 mM EDTA

RECOVERY OF DNA FROM LOW MELTING POINT AGAROSE GEL:

1. Cut DNA band of interest from the low melting point agarose gel under a long-wave UV light, and transfer the agarose piece into a tared eppendorf tube.
2. Determine the weight of the agarose piece and add 0.1 volumes of 10 Agarase Buffer. Incubate the tube for 15 min at 65°C until the agarose is completely molten. A clear, nonviscous solution is required to be sure that all cleavage sites are accessible to the agarase.
3. Cool down the molten agarose to 42-45°C and add 1 unit agarase per 100 mg of agarose (100 μ l 1% agarose in Tris-acetate).
Note: When the percentage of agarose is higher than 1%, the units of agarase have to be adjusted in proportion. When using Tris-borate gel electrophoresis buffer, a two-fold amount of agarase should be used; otherwise, the incubation time has to be prolonged.
4. Carefully mix the solution and incubate for 1 hour at 42-45°C.

5. Add 0.1 volume of 3 M sodium acetate, pH 5.5, to the molten agarose solution and incubate 15 min on ice.
6. Centrifuge for 15 minutes at 2-8°C to pellet the oligosaccharides.
7. Precipitate the nucleic acids from the supernatant with 3 volume of ice-cold ethanol as usual.

FOR RESEARCH USE ONLY! Not to be used in human.

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- Mammalian Cell Extraction Kit
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