

β-Agarase

Product Description:

Cambrex β-Agarase, an enzyme preparation derived from *Pseudomonas atlantica*, will digest the polysaccharide backbone of agarose [3, 6-anhydro-α-L-galactopyranosyl-(1→3)-D-galactose] to alcohol soluble neoagaro-oligosaccharides.^{1, 2, 3} DNA or RNA electrophoresed in a low-melting-temperature agarose gel can be quantitatively recovered after the gel is melted and digested with this enzyme preparation.^{4, 5} The remaining alcohol soluble neoagaro-oligosaccharides will not gel or interfere with subsequent DNA manipulations such as cloning, labeling, restriction digestion, or sequencing. Cambrex β-Agarase is free of any detectable DNase, RNase, or phosphatase activity. β-Agarase is particularly useful for recovering large DNA (>10 kb) which could be sheared by other methods of recovery.

Contaminating Enzyme Activity:

Cambrex β-Agarase is tested for the presence of contaminating enzyme activity by incubating 30 units of β-Agarase with 1 μg each of the substrates listed below under the following conditions: 16 hours at 37°C in 50 μl of a buffer consisting of 33 mM Tris/acetate, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, and 100 μg/ml BSA, pH 7.8.

DNase: There is no change in the electrophoretic migration of: *Hind* III digested λ phage DNA; super coiled plasmid DNA; a 5' phosphorylated 40 base oligomer; or a 5'-OH 40 base oligomer indicating the absence of any DNase activity.

RNase: There is no change in the electrophoretic migration of an *in vitro* generated SP6 RNA polymerase transcript indicating the absence of any RNase activity.

Phosphatase: There is no degradation of p-nitrophenyl-phosphate indicating the absence of any phosphatases.

Storage:

The enzyme is stable when stored at -20°C.² β-Agarase is supplied in a storage buffer consisting of 50% glycerol, 50 mM Tris/HCl, 100 mM NaCl, and 0.1% Triton® X-100 surfactant at pH 7.5

Unit Definition:

One unit of β-agarase will completely digest 200 mg of a molten 1% SeaPlaque® GTG® Agarose Gel prepared in 1X β-Agarase buffer in one hour at 40° C. Similar activities are obtained for other low-melting-temperature agaroses such as NuSieve® GTG Agarose.

Concentration: 1 unit per μl

β-Agarase Buffer:

Supplied as a 50X concentrate.
1X concentration is: 40 mM Bis Tris/HCl, 40 mM NaCl, 1 mM EDTA (pH 6.0).

Agarose Digestion:

Before you begin:

- Set one water bath or heating block for 70°C. Set another water bath or heating block for 45°C.
- Electrophorese your DNA or RNA sample in a low-melting-temperature agarose gel (SeaPlaque, SeaPlaque GTG, or NuSieve GTG Agarose) prepared in TAE, TBE, or MOPS buffer.
- Place no more than 200 mg of an agarose gel slice into a 2 ml microcentrifuge tube.

Basic Method:

1. Equilibrate the gel slice with 10 volumes of 1X β-Agarase buffer for 60 minutes at room temperature, then discard the buffer. See Application Notes 1.
2. Melt the gel slice at 70°C (approximately 15 minutes). Make sure that the gel slice is completely melted as the enzyme will not digest agarose in the gelled state.
3. Cool the melted gel to 45°C and add 1 unit of β-Agarase for 200 mg (approximately 200 μl) of 1% agarose gel, mix and incubate at 45°C for 60 minutes.
4. Add proportionally more or less of the enzyme preparation with larger or smaller gel slices. Also, add proportionally more enzyme to agarose gel concentrations higher than 1%. For example, 3 units of β-Agarase should be used to digest 200 mg of a 3% agarose gel.

NOTE: The enzyme preparation retains full activity for several hours at 45°C. However it will gradually lose activity upon longer incubations at 45°C. For this reason we recommend incubation at 40°C for overnight digestions.

Fast Method: If you wish to save time use the fast equilibration method.

1. Melt the gel slice at 70°C, (approximately 15 minutes). Make sure the gel slice is completely melted as the enzyme will not digest agarose in the gelled state.
2. Add 2 μl of 50X β-Agarase buffer to every 100 μl of gel solution.
3. Mix.
4. Cool the melted gel to 45°C.
5. **For TAE gels or MOPS gels:** add 2 units of β-Agarase for 200 mg (approx. 200 μl) of 1% agarose gel.
For TBE gels: add 7 units of β-Agarase for 200 mg (approx. 200 μl) of 1% agarose gel. Add proportionally more or less enzyme with larger or smaller gel slices, or with agarose concentrations higher or lower than 1%. For example, 21 units of β-Agarase should be used to digest 200 mg of a 3% agarose TBE gel. (approx. 200 μl).

DNA Recovery and Purification:

For Fragments ≤30 kb

- Make sure the solution has no particles in it; it should look clear.
- Place at -20°C for 30 minutes.
- Spin at 4°C for 15 minutes.
- Remove the supernatant and place it into a clean microcentrifuge tube.

At this point the sample can be concentrated without ethanol precipitation by centrifugation in a molecular weight-cutoff-spin column.

To Ethanol Precipitate:

- Add ammonium acetate to the supernatant to a final concentration of 2.5 M. The use of ammonium acetate rather than sodium acetate decreases the likelihood of co-precipitation of agarose-oligosaccharides with the nucleic acid.
- Add 2 to 3 volumes of 100% ethanol.
If the DNA is ≤0.05 µg/ml add 10 µg of carrier RNA and precipitate at room temperature for 24 hours. See Applications Note 2.
If DNA is ≥0.05 µg/ml precipitate at room temperature for 30 minutes.
- Collect the precipitate by centrifugation. Centrifugation of the precipitated DNA should be done for a full 30 minutes at maximum speed (12,000 to 16,000 x g). The 30 minutes centrifugation time is important for smaller fragments and lower concentrations.

For Fragments ≥30 kb

- The DNA/β-Agarase solution may be used directly without precipitation for subsequent enzymatic manipulations. Alternately the sample can be concentrated without ethanol precipitation by centrifugation in a molecular weight-cutoff-spin column.⁽⁹⁾

Application Notes:

1. The enzyme preparation has maximum activity between pH 6 and 7 and is relatively unaffected by salt concentrations between 0.1 M and 0.25 M.⁽²⁾ The equilibration of the gel slice with β-Agarase buffer is necessary to provide the enzyme with optimal buffer conditions. This equilibration is more important for gels prepared in TBE than in TAE buffer due to the greater buffering capacity of TBE buffer.
2. For efficient recovery of small nucleic acids (<500 bp) and/or very dilute samples (>0.5 µg/ml), we recommend that either carrier tRNA or nuclease free glycogen be added. In addition, overnight precipitation at room temperature can be helpful.^(7, 8)
3. Please note that polynucleotide kinase is inhibited by ≥7 mM ammonium ion.⁽⁶⁾ When phosphorylating your DNA sample with this enzyme, sodium acetate may be a better choice than ammonium acetate.
4. When recovering RNA from agarose gels, be sure that any denaturants in the gel (e.g. formaldehyde or glyoxal) are completely removed prior to adding the β-Agarase. These denaturants will interfere with enzyme activity. They can be removed by equilibrating the gel slice in the β-Agarase buffer prior to adding the enzyme preparation.

References:

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Ordering Information

| Catalog No. | Description | Size |
|--------------------|--------------------|-------------|
| 58001 | β -Agarase | 100 units |
| 58005 | β -Agarase | 500 units |

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Not for use in Diagnostic Procedures.

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The Sourcebook

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