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Excellence in Molecular Biology

# Agarose Gel-Digesting Enzyme

## (Thermostable β-Agarase – Cambio's recommended replacement for Epicentre GELase™)

CA-1725-030 30 units (1U/ul)

CA-1725-300 300 units (1U/ul)

### Introduction

**Cambio's Agarose Gel-Digesting Enzyme (Thermostable β-Agarase)** is derived from a heat-resistant microorganism collected from the deep sea and developed for the simple and quantitative recovery of intact DNA and RNA from low-melting-point (LMP) agarose gels. Cambio's Agarose Gel-Digesting Enzyme digests the polysaccharides in molten agarose to neoagaro-oligosaccharides, rendering the agarose solution incapable of forming a gel again. This property allows Agarase to be used for extracting nucleic acid from agarose gel to yield a clear liquid that does not become viscous or gel on cooling to 0°C.

As the oligosaccharide digestion products are soluble in ethanol, nucleic acids can be precipitated directly from the solution of digested agarose. Nucleic acids of any size, from less than 50 nucleotides to megabase DNA can be easily purified intact and in high yields. Nucleic acids recovered using Agarose Gel-Digesting Enzyme procedure are ready for use in genomic DNA cloning, restriction mapping, sequencing, labeling, amplification or other molecular biological manipulations.

### Advantages and key features

This product is a thermostable agarase derived from a heat-resistant microorganism and has excellent heat resistance compared to conventional agarose.

- The advantages of nucleic acid extraction by agarase include the following:
- No hazardous reagents are used
- Relatively large DNA fragments can be recovered without damage.
- No Organic extractions necessary.

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### Properties

- Manipulations are simple and quick.
- The optimum reaction temperature is high, and this product can be used for standard agarose.
- The degraded gel solution can be used as-is for reactions such as cloning and restriction enzyme reactions. Large DNA fragments can be recovered with minimal shearing.

**Product specifications**

**Composition**：**Agarose Gel-Digesting Enzyme**（30 units / 300 units）×1
**Activity**： 1 unit/ μl
**Storage Conditions**： 50 mM NaCl, 20 mM Tris-HCl (pH 7.5)
**Definition of Units**：One unit is defined as an enzyme activity that produces reducing sugar equivalent amount of 1 μmol D-galactose from agarose gel per 1 min at 60°C.

**Storage Temperature**
Keep refrigerated at 2-10°C

## Low melting point agarose protocol

Electrophorese DNA on a low melting point agarose / TAE gel.[1]

Place 200 mg (～200 μl) of excised LMP agarose gel slice in a microtube

Heat for 5-10 min at 55-65°C [1] to completely melt the agarose gel.

After confirming that the gel slice has melted, add 6 units (6 μl) of Agarose Gel-Digesting Enzyme and mix.

Incubate at 50-65°C for 5-10 min to degrade agarose.[2,3]

Allow to stand on ice and visualise by eye that the solution does not re-coagulate. [4,5]
(Perform alcohol precipitation or phenol/chloroform step if required)

**Important Notes:**

*1. The melting point of the gel depends on the type of agarose and buffer used. Low melting point agarose and TAE buffer are recommended.*

*2. Agarose Gel-Digesting Enzyme demonstrates the maximum activity at 50-60°Cand also maintains 10% or higher activity after heating at 70°C for 30 min.*

*3. The reaction time varies depending on the amount of agarose gel block and Agarose Gel-Digesting Enzyme being added.*

*4. When recovered DNA solution is to be used for re-electrophoresis, the enzyme should be inactivated by heat treatment (90°C, 5 min) or by a phenol/chloroform treatment.*

*5. If undegraded agarose gel is still present, remove the gel by centrifuge, or restart the reaction from the start of the protocol. If necessary, perform alcohol precipitation/purification.*

# Troubleshooting

## Problems and counter measures

### Undegraded gel remaining

Insufficient reaction of Agarose Gel-Digesting Enzyme may be the cause.
1. Add Agarose Gel-Digesting Enzyme after confirming that the gel is completely melted in step (2) (gel-dissolving reaction) of the basic protocol by pipetting, etc.
2. Increase the amount of Agarose Gel-Digesting Enzyme to be added.
3. Extend the reaction time of the gel degradation after adding Agarose Gel-Digesting Enzyme.

### Low purity of recovered DNA

High molecular weight degradation products by agarose may remain.
1. Completely degrade the gel.
2. Precipitate the undegraded substance by centrifugation and use the supernatant as the DNA solution.
3. Perform alcohol precipitation/purification. Perform phenol/chloroform treatment for further purification.

### Thinning of the gel around the well in re-electrophoresis of recovered DNA

Agarose Gel-Digesting Enzyme may be persisting.
1. Inactivate by performing a heat treatment at 90°C or higher for 5 min or longer.
2. Perform phenol/chloroform treatment.

# FAQs

Q1. What is the degree of DNA recovery?
A1. In principle, all DNA in the excised gel is recovered.

Q2. What is the final volume of the reaction solution?
A2. When 200 mg of agarose gel is used, the final volume of the solution is about 200 µl.

Q3. How can the reaction solution be concentrated?
A3. Perform alcohol precipitation.

Example: Add 0.1 volume of 3 M sodium acetate (pH 5.2) to the obtained DNA solution (degraded gel solution) and mix. Add 0.8-1 volume of isopropanol (or 2.5 volumes of ethanol), mix, leave standing at room temperature for 2-10 min, centrifuge (12 K X g, 10 min) and remove the supernatant. Add approx. 1 ml of 70% ethanol to the tube containing the precipitated DNA, centrifuge (12 K X g, 5 min) and remove the supernatant. Leave the precipitate at room temperature for 10 min to dry and then dissolve into an appropriate amount of TE buffer.

Q4. Can high molecular weight DNA fragments be recovered?
A4. We have confirmed that large DNA fragments can be recovered with much less physical shearing (confirmed by recovering DNA fragments of tens of bp to 170 kb) with Agarose Gel-Digesting Enzyme. However, the bigger the size of the DNA to be manipulated, the more difficult it is to separate by agarose gel electrophoresis, and the more prone it will be to shearing by manipulations such as pipetting.

Q5. Can the reaction solution be used for PCR reactions?
A5. We have confirmed that the solution can be used for PCR reactions by adding not more than 1/8 volume of the agarose solution degraded by Agarose Gel-Digesting Enzyme. Example: When the volume of the PCR reaction solution is 20 µl, add 2.5 µl or less of the degraded agarose solution.

Q6. Can the reaction solution be used for cloning?
A6. We have confirmed that DNA solution recovered by Agarose Gel-Digesting Enzyme can be used as-is for ligation and transformation.

Q7. Can the reaction solution be used for in vitro transcription reactions?
A7. We have confirmed that DNA solution recovered with Agarose Gel-Digesting Enzyme can be used as-is for in vitro transcription reactions.

Q8. Can the reaction solution be used for sequencing?
A8. We have confirmed that DNA solution recovered by Agarose Gel-Digesting Enzyme can be used as-is for Sanger sequencing. However, if high-concentration/high molecular weight degraded products of agarose are carried over, there may be problems depending on the model of the analytical instrument. High molecular weight degraded products of agarose gel can be removed with a phenol/chloroform treatment.

Q9. Can this product be used for extracting RNA from denaturing agarose gels?
A9. It can be used, but since formaldehyde included in denaturing agarose gel block inhibits the enzyme activity, the amount of the enzyme to be added needs to be increased. Example: After melting 100 mg of 1.5% denaturing agarose gel slice at 90°C for 5 min, agarose is degraded by adding 8 µl (8 units) of the enzyme and incubating at 60°C for 10 min.