

GELase™ Agarose Gel-Digesting Preparation GELase™ 50X Reaction Buffer

Cat. Nos. G09050, G09100, G09200, G31050, G31200, G191ML, and G195ML

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1. Introduction

GELase™ Agarose Gel-Digesting Preparation was developed for the simple and quantitative recovery of intact DNA and RNA from low-melting-point (LMP) agarose gels. GELase Preparation digests the polysaccharides in molten agarose 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^{1,2} can be easily purified intact and in high yields. Nucleic acids recovered using the GELase procedure are ready for use in genomic DNA cloning,³⁻⁶ restriction mapping, sequencing,^{7,8} labeling, amplification^{9,10}, or other molecular biological manipulations.¹¹ Organic extractions are unnecessary.

GELase Preparation (available at a concentration of 0.2 U/μl or 1.0 U/μl) is provided with two protocols. In the High-Activity Protocol, the electrophoresis buffer is replaced with GELase Buffer before melting and digestion, allowing the use of less enzyme. Alternatively, in the Fast Protocol, GELase Buffer is added directly to the gel slice without first removing the electrophoresis buffer, allowing faster recovery of the nucleic acids.

2. Product Specifications

Storage: Store only at -20°C in a freezer without a defrost cycle.

Storage Buffer: GELase Preparation is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 100 mM NaCl, 0.1% Triton® X-100, and 1 mM dithiothreitol.

Unit Definition: One unit of GELase Preparation digests 600 mg of molten 1% LMP-agarose in 1X GELase Buffer in 1 hour at 45°C.

50X GELase Buffer: 2 M Bis-Tris (pH 6.0) and 2 M NaCl.

Contaminating Activity Assays: GELase Preparation is free of detectable DNA exo- and endonuclease and RNase activities.

3. Related Products

The following products are also available:

- EpiFOS™ Fosmid Library Production Kit
- pWEB™ and pWEB-TNC™ Cosmid Cloning Kits
- pIndigoBAC-5 (Cloning Ready) BAC Vectors
- FailSafe™ PCR System
- MasterAmp™ AmpliTherm™, *Tfl*, *Taq*, and *Tth* Thermostable DNA Polymerases
- Fast-Link™ DNA Ligation and Screening Kits
- T4 DNA Ligase
- Ampligase® Thermostable DNA Ligase

4. General Considerations

1. **Type and Source of Agarose:** Generally, any high-quality LMP agarose developed for molecular biology, that remains molten at 45°C, is suitable.

Note: Agaroses that melt at higher temperatures are also substrates for GELase enzyme. However, exposure of nucleic acids to the high temperatures required to melt agarose will denature double-stranded DNA and degrade RNA. If the use of a high-melting-point agarose is necessary for resolution and nondenatured DNA is required, cut a hole in the gel below the band of interest and fill it with LMP agarose. Transfer the nucleic acid into the LMP agarose plug by electrophoresis and treat the LMP-agarose plug with GELase enzyme.¹¹

2. **Gel Electrophoresis Conditions:** The presence of borate ions can detrimentally affect the activity of many enzymes (including GELase enzyme). Therefore, we recommend using TAE for gel electrophoresis if the recovered DNA is to be used in subsequent applications without purification or concentration.
3. **Choosing between the Fast and High-Activity Protocols:** Two protocols are described that differ by the inclusion of a buffer exchange step. The High-Activity Protocol achieves optimal enzymatic activity through exchange of the electrophoresis buffer with GELase Buffer, thereby allowing the use of less enzyme. Conversely, the Fast Protocol allows recovery of nucleic acids in the shortest time possible by omission of the buffer exchange step, but more enzyme is required. Use the Fast Protocol when purifying very small nucleic acids (<100 nt or bp) that might diffuse out of the gel during the buffer exchange step. Use the High-Activity Protocol when isolating nucleic acids from gels containing denaturants such as formaldehyde, glyoxal, or NaOH, or to conserve enzyme, thereby decreasing reaction cost.
4. **Calculating the Amount of Enzyme To Use in a Reaction:** Determine the amount of enzyme to use in a reaction by both the size of the gel slice and the percent agarose. Furthermore, the presence of electrophoresis buffer salts in the digestion reaction (i.e., when using the Fast Protocol, see above) requires use of additional enzyme. For example, if performing the High-Activity Protocol, 1 unit of GELase enzyme will digest 600 mg of 1% agarose gel in 60 minutes. If performing the Fast Protocol, in 60 minutes, 1 unit of enzyme will digest: 400 mg of a 1% gel containing TAE buffer; 80 mg of a 1% gel containing TBE buffer; 300 mg of a 1% gel containing MOPS buffer; and 120 mg of a 1% gel containing phosphate buffer. Use a simple ratio to calculate the amount of GELase enzyme needed for a given reaction:

Fast Protocol for a 475-mg 1.5% gel slice in TAE, 60-minute digest.

$$\frac{(x \text{ GELase Units})}{(475 \text{ mg}) (1.5\%)} = \frac{(1 \text{ GELase Unit})}{(400 \text{ mg}) (1.0\%)} \quad X = 1.8 \text{ U}$$

High-Activity Protocol for a 475-mg 1.5% gel slice, 60-minute digest.

$$\frac{(x \text{ GELase Units})}{(475 \text{ mg}) (1.5\%)} = \frac{(1 \text{ GELase Unit})}{(600 \text{ mg}) (1.0\%)} \quad X = 1.2 \text{ U}$$

5. Purifying and Concentrating Nucleic Acids Following GELase Enzyme

Digestion: Nucleic acids recovered from agarose gels are ready to use in many applications without further purification, provided the concentration of the nucleic acid is adequate (e.g., restriction digestion, ligation, amplification). Nevertheless, residual electrophoresis buffer salts and oligosaccharides generated by digestion of the agarose may decrease the efficiency of further manipulations (e.g., transformation or electroporation). These salts and oligosaccharides can be removed by precipitating the nucleic acids with ethanol if the nucleic acids are less than 30 kb in length. We do not recommend ethanol precipitation of high-molecular-weight DNA, as centrifugation will shear the DNA.¹² For larger DNA molecules, you can exchange salts by using microconcentrators¹³ or dialysis.¹⁴ Ammonium ions strongly inhibit T4 Polynucleotide Kinase (PNK); therefore, use 3 M sodium acetate in place of ammonium acetate to precipitate nucleic acids if using T4 PNK in subsequent manipulations.

5. Protocols for the Digestion of Agarose Gel Slices

A. Fast Protocol

1. Cut out the agarose band and trim excess agarose. Limit exposure of the gel slice to ultraviolet (UV) light to minimize the formation of pyrimidine dimers. The presence of ethidium bromide will not adversely affect digestion of the gel slice.
2. Weigh the gel slice in a tared tube.
3. **Note:** GELase Buffer is inhibitory to lambda packaging reactions. If preparing cosmid or fosmid libraries from the DNA, do not perform this step; go directly to Step 4. Add 1 µl of 50X GELase Buffer to the tube for each 50 mg of gel (1 µl molten agarose = 1 mg gel).
4. Thoroughly melt the gel slice by incubating at 70°C for >3 minutes for each 200 mg of gel. Cut gel slices >200 mg into smaller pieces to facilitate melting. To monitor melting, pipet the molten agarose; if the suspension does not pipet easily, continue incubating at 70°C for a few more minutes.

Note: Temperatures higher than 70°C can denature DNA and degrade RNA.

5. Transfer the molten agarose immediately to 45°C and equilibrate 2 minutes for each 200 mg of gel. The GELase enzyme loses activity at temperatures greater than 45°C.
6. Calculate the amount of GELase enzyme required to digest the gel slice at 45°C (see General Considerations). Adjust the digestion time by altering the amount of enzyme in the reaction. For example, the digestion conditions for a 200-mg, 1% gel slice containing TAE buffer are:

<u>Digestion Time</u>	<u>Units of Enzyme</u>
15 minutes	2.0
30 minutes	1.0
1 hour	0.5

7. After GELase digestion is complete, check the DNA concentration by fluorimetry or by running an aliquot on an agarose minigel against known concentration standards. In many cases, the DNA is concentrated enough to be added directly to a ligation reaction,³⁻⁶ restriction endonuclease digestion, or PCR. If the concentration of the DNA is too low, it can be concentrated by NH₄OAc/ethanol precipitation as described in Steps 8-11.

8. If concentrating the nucleic acid by ethanol precipitation (see General Considerations), add an equal volume of fresh 5 M NH_4OAc (pH 7.0) (to a final concentration of 2.5 M) to the digested agarose and mix well. Add four volumes of room-temperature ethanol.
Note: *Using cold ethanol will coprecipitate oligosaccharides.*
Note: *The molarity of the NH_4OAc solution is critical for efficient recovery of the nucleic acids. Crystalline NH_4OAc is quite hygroscopic; using wet crystals can dramatically decrease the molarity of a solution. The oligosaccharides produced by GELase digestion are more soluble in ethanol in the presence of ammonium; using other salts for ethanol precipitation may cause coprecipitation of the oligosaccharide digestion products.*
9. If precipitating >500 ng of a nucleic acid >500 bp in length, pellet the nucleic acid by centrifugation for 15 minutes at room temperature. If the nucleic acid concentration is <0.5 $\mu\text{g}/\text{ml}$, incubate overnight at room temperature to improve recovery. When isolating <500 ng of DNA or when isolating DNA molecules <500 bp long, pellet the nucleic acids by centrifugation for 30 minutes at room temperature.
Note: *Carrier RNA may be used to enhance the efficiency of precipitation. Do not use glycogen to enhance ethanol precipitation, as it may cause oligosaccharide contamination of the pellet.*
10. Carefully remove the supernatant with a pipet. Do not decant the supernatant as the nucleic acid pellet is easily dislodged.
11. Wash the pellet with 70% ethanol and carefully remove the supernatant with a pipet.

B. High-Activity Protocol

1. Cut out the agarose band and trim excess agarose. Limit exposure of the gel slice to UV light to minimize the formation of pyrimidine dimers. The presence of ethidium bromide will not adversely affect digestion of the gel slice.
2. Weigh the gel slice in a tared tube.
3. **Note:** *GELase Buffer is inhibitory to lambda packaging reactions. If preparing cosmid or fosmid libraries from the DNA, do not perform this step, go directly to Step 4.*
Exchange the electrophoresis buffer in the gel slice with 1X GELase Buffer (dilute the 50X Buffer with sterile water) by adding 3 μl of 1X GELase Buffer for every 1 mg of gel. Incubate at room temperature for 1 hour, then remove all of the 1X GELase Buffer.
4. Thoroughly melt the gel slice by incubating at 70°C for >3 minutes for each 200 mg of gel. Cut gel slices >200 mg into smaller pieces to facilitate melting. To monitor melting, pipet the molten agarose; if the suspension does not pipet easily, continue incubating at 70°C for a few more minutes.
Note: *Temperatures higher than 70°C denature DNA and degrade RNA.*
5. Transfer the molten agarose immediately to 45°C and equilibrate 2 minutes for each 200 mg of gel. The GELase enzyme loses activity at temperatures greater than 45°C.

6. Calculate the amount of GELase enzyme required to digest the gel slice at 45°C (see General Considerations). Adjust the digestion time by altering the amount of enzyme in the reaction. For example, the digestion conditions for a 200-mg, 1% gel slice are:

<u>Digestion Time</u>	<u>Units of Enzyme</u>
15 minutes	1.3
30 minutes	0.66
1 hour	0.33

7. After GELase digestion is complete, check the DNA concentration by fluorimetry or by running an aliquot on an agarose minigel against known concentration standards. In many cases, the DNA is concentrated enough to be added directly to a ligation reaction,³⁻⁶ restriction endonuclease digestion, or PCR. If the concentration of the DNA is too low, it can be concentrated by NH₄OAc/ethanol precipitation as described in Steps 8-11.
8. If concentrating the nucleic acid by ethanol precipitation (see General Considerations), add an equal volume of fresh 5 M NH₄OAc [pH 7.0] (to a final concentration of 2.5 M) to the digested agarose and mix well. Add four volumes of room-temperature ethanol.

Note: Using cold ethanol will co-precipitate oligosaccharides.

Note: The molarity of the NH₄OAc solution is critical for efficient recovery of the nucleic acids. Crystalline NH₄OAc is quite hygroscopic; using wet crystals can dramatically decrease the molarity of a solution. The oligosaccharides produced by GELase digestion are more soluble in ethanol in the presence of ammonium; using other salts for ethanol precipitation may cause coprecipitation of the oligosaccharide digestion products.

9. If precipitating >500 ng of a nucleic acid >500 bp in length, pellet the nucleic acid by centrifugation for 15 minutes at room temperature. If the nucleic acid concentration is <0.5 µg/ml, incubate overnight at room temperature to improve recovery. When isolating <500 ng of DNA or when isolating DNA molecules <500 bp long, pellet the nucleic acids by centrifugation for 30 minutes at room temperature.

Note: Carrier RNA may be used to enhance the efficiency of precipitation. Do not use glycogen to enhance ethanol precipitation, as it may cause oligosaccharide contamination of the pellet.

10. Carefully remove the supernatant with a pipet. Do not decant the supernatant as the nucleic acid pellet is easily dislodged.
11. Wash the pellet with 70% ethanol and carefully remove the supernatant with a pipet.

6. Troubleshooting Agarose Digestion Reactions

Undigested gel remains

- 1) **Ensure that the gel slice is thoroughly melted.** Increase the gel slice melting time and monitor melting by pipetting the molten agarose. Do not flick or invert the tube, which may cause the molten agarose to solidify on the side or top of the tube. Transfer the molten agarose immediately to 45°C.

- 2) **Increase the amount of enzyme in the reaction.** Calculate the amount of enzyme required using the relationship outlined in General Considerations. Note that additional enzyme is required if performing the digestion in any buffer other than 1X GELase Buffer. For more efficient digestion, cut large pieces of gel (>1 g) into smaller pieces and digest in separate tubes. Alternatively, digest large pieces of gel in a single tube and occasionally stir the suspension (while keeping the tube at 45°C) to ensure complete access of the enzyme to the substrate.
- 3) **Increase the length of digestion.** Check that the digestion was performed at the appropriate temperature, and that the tube was completely submerged in the water bath. Increase the time of digestion by 15-30 minutes.
- 4) **Equilibrate the molten agarose at 45°C before addition of GELase enzyme.** GELase enzyme quickly loses activity at temperatures above 45°C. Ensure that the molten agarose equilibrates to 45°C before adding the enzyme.
- 5) **Resuspend the nucleic acids and recover by centrifugation.** If the amount of gel in the nucleic acid pellet is small, redigestion may be unnecessary. Resuspend the nucleic acid in an appropriate volume of TE or water and centrifuge the tube for 2-3 minutes in a microcentrifuge. Transfer the nucleic acid to a clean tube.
- 6) **Check the type and age of the agarose.** Agarose may degrade with time (>5 years) resulting in polysaccharides that are resistant to cleavage. In addition, agarose-acrylamide mixtures are not efficiently digested by the GELase enzyme.

Recovery of nucleic acids is poor

- 1) **Ethanol-precipitate overnight at room temperature.** The recovery of small DNA and RNA molecules (<500 bp) and small quantities of nucleic acids (<0.5 µg/ml) is improved by ethanol precipitation overnight at room temperature. Pellet the nucleic acids by centrifugation for 30 minutes at room temperature.
- 2) **Remove ethanol from nucleic acid pellets carefully.** Nucleic acids precipitated in the presence of ammonium acetate form loose pellets that are easily dislodged from the side of the tube. Do not pour off the ethanol, but carefully remove the supernatant using a pipet.
- 3) **Prepare fresh ammonium acetate solution.** The molarity of the ammonium acetate solution is critical for efficient precipitation of the nucleic acid. We recommend preparing ammonium acetate solutions by the addition of ammonium hydroxide to glacial acetic acid to pH 7.0, followed by filter-sterilization. Store the solution at 4°C.

7. References

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