

Frequently Asked Questions

PowerPrep™ GEL EXTRACTION KITS

Q: What are the minimum and maximum fragment sizes that can be purified?

A: For both the PowerPrep™ Express and Matrix Gel Extraction Kits, the lower limit is 80 nt. For the Express Gel Extraction Kits the upper limit is 10 kb. For very large fragments (up to 50 kb) the Matrix Gel Extraction Kit is the recommended choice. Outside of these limits, recovery will be reduced by 50% or more.

For single-stranded DNA and RNA, the lower limit is about 200 nt.

Q: What types of agarose can be used?

A: Both regular and low-melting agarose can be used. A slightly different protocol must be used for gel concentrations of greater than 2%.

Q: Why isn't my gel solubilized?

A: This can occur for several reasons:

- The incorrect ratio of Gel Solubilization Buffer to agarose mass was used. For gel concentrations up to 2%, use 300 µl of Gel Solubilization Buffer for every 100 mg of gel. For gel concentrations greater than 2%, use 600 ml of Gel Solubilization Buffer for every 100 mg of gel.
- The solubilization step was carried out below 50°C.
- The solubilization tube was not vortexed thoroughly every three minutes.
- The solubilization step was not carried out long enough.
- Was the mass of the agarose gel slice greater than 100 mg? If so, mince the agarose to speed solubilization.

Q: Why is there a precipitate in my sample after I added Gel Solubilization Buffer?

A: If your sample contains SDS or potassium ions, a precipitate may form. After solubilization is complete, spin down the precipitate and transfer the supernatant to the spin column.

Q: Why is there gel in my eluted sample?

A: The agarose gel must be thoroughly dissolved before processing. Frequent and vigorous mixing is critical during the dissolution. Also be sure to use sufficient amounts of Buffer L1 to dissolve the gel. Mincing of the gel is not generally required but will speed the dissolution process.

Q: Subsequent enzymatic reactions did not work. Why?

A: There are several reasons why enzymatic reactions can be inhibited:

- Many enzymes including restriction endonucleases and ligases are inhibited by small amounts of agarose and/or perchlorate contaminants. This is not normally a problem. If it is, however, one can use the DNA without re-purification by increasing the amount of enzyme used in the digest or ligation or by increasing the digestion incubation time. Also, you may use less DNA in your digest/ligation with the same total volume and the same amount of restriction enzyme.
- There may have been residual ethanol in the eluted fragment. Be sure to thoroughly centrifuge to remove the Wash Buffer, discard the Wash Buffer and use a fresh tube to collect the eluted DNA. For applications that are very sensitive to ethanol, let the open spin column stand for 15 minutes at room temperature to let any excess ethanol evaporate.
- The washing steps may not be as efficient as they should be. Under these circumstances, there may be trace amounts of perchlorate in the eluate. To avoid this, extend the centrifugation times to 5 minutes and wash 2 times with Wash Buffer.
- Be sure to perform the optional wash step if you are using higher concentrations of agarose and when you are adding more than 250 mg to the cartridge.
- If applications are sensitive to EDTA, elute with water (pH 7.5 – 8.5), or with 10 mM Tris, pH 8.0 without EDTA.

Q: May I elute in water?

A: Yes, but be sure that you are using a very clean water source and that the pH of the water is pH 7.5 – 8.5.

Q: Can I use this kit to elute supercoiled DNA?

A: If using the PowerPrep™ Express Gel Extraction Kit, supercoiled DNA can be eluted from the membrane only when the Wash Buffer contains 50-55% ethanol. Supercoiled DNA will not be eluted from the membrane using the standard Wash Buffer that contains 70% ethanol. Supercoiled DNA cannot be used with the PowerPrep™ Matrix Gel Extraction Kit.

Q: My yield was lower than expected. Why?

A: There are several reasons low yields can occur:

- More than 400 mg of agarose was loaded per cartridge. This will decrease the performance of the cartridge.
- An incorrect ratio of Solubilization Buffer to gel was used. For gel slices of more than 100 mg, the volume of Solubilization Buffer must be scaled up accordingly.

- Was ethanol added to the Wash Buffer? Ethanol is necessary to keep DNA bound to the silica membrane.
- Was the bottle with Buffer L2 kept tightly closed when not in use? If evaporation reduces the ethanol content in Buffer L2, recovery of DNA will be increasingly poor.
- The gel was not completely solubilized before it was added to the column. Make sure to dissolve at 50°C and to mix every three minutes.
- The DNA was not completely eluted. Make sure to use TE prewarmed to 65-70°C.
- If using the PowerPrep™ Matrix Gel Extraction Kit to purify DNA fragments of larger than 10 kb verify that the elution step was increased by 15 minutes.
- Was the DNA supercoiled? To elute supercoiled DNA from the membrane, the ethanol concentration in the Wash Buffer must be 50-55%. Supercoiled DNA will not be eluted from the membrane using the standard Wash Buffer that contains 70% ethanol. Supercoiled DNA cannot be used with the PowerPrep™ Matrix Gel Extraction Kit.
- Was the kit stored at 4°C? The PowerPrep™ Express Gel Extraction Kit should be stored and used at room temperature. Use of cold buffers may reduce yield.

Q: Why did I get multiple bands on post-extraction gel analysis?

A: This can be caused by attempting to extract partially single-stranded bands. The most likely cause of this is running the gel on which the fragment to be purified is separated too fast. The heat generated during electrophoresis at high currents may be sufficient to cause partial denaturation of the fragments, which results in poor yields during purification and in multiple bands when the eluted DNA is electrophoresed.

AT-rich DNA may be denatured during the 50°C incubation to dissolve the gel slices. If this happens, solubilize the gel at 37°C for 20-30 minutes with repeated vortexing.

For further technical assistance please contact our Technical Service Department at technical.support@marligen.com or (301)-874-4990.