

Frequently Asked Questions

PowerPrep™ HP PLASMID PURIFICATION KITS

Q: I did not recover any DNA. Why?

A: The DNA pellet from precipitation with isopropanol is easily dislodged when washing with 70% ethanol. It is best to pipet the isopropanol supernatant and ethanol wash to remove from the pellet. This is a particular problem for minipreps, where the DNA pellet is so small. This same problem exists for other manufacturers' DNA miniprep kits.

Q: My yield was lower than expected. What could be the cause?

A: There are several reasons lower yields can occur:

- Make sure the binding of the plasmid is being done at RT. Temperature affects the pH of the binding solution.
- Make sure all other solutions were also warmed to RT for optimal yield.
- Verify that the centrifugation immediately following the neutralization step was not done at 4°C. If it was, the supernatant MUST be warmed to RT before loading onto the column.
- The 1:1:1 ratio of buffers E1/E2/E3 has not been kept precisely. A surplus of solution E3 will exceed the optimal salt concentration for DNA binding, thus leading to decreased DNA yield.
- Low copy number plasmids give lower yields.
- If all of the media was not removed at the cell harvesting step, the pH of the subsequent step can be affected and reduce yield.
- The cell pellet was not thoroughly resuspended before lysis.
- The purified DNA pellet was overdried after isopropanol precipitation and ethanol wash making it difficult to resuspend the pellet. Air dry only.
- Pellets can be easily lost during the alcohol precipitation and wash steps. BE CAREFUL. It is best to pipet off the alcohol solutions rather than pouring them off, as the pellets tend to be slippery.
- After lysis and neutralization, the lysate is not homogeneous. After addition of solutions E1, E2, and E3, cells must be mixed thoroughly by inverting the tubes until a homogeneous phase is obtained. Otherwise, cells will not be lysed completely and the plasmid DNA will stick to bacterial debris. In the case of bigger bacterial cell pellets, more vigorous shaking may be required to achieve homogeneity. However, DO NOT VORTEX, as vortexing will release chromosomal DNA.

Q: Why can't I dissolve all of my DNA after precipitation?

A: Occasionally there is insoluble material (probably resin fines) in the precipitated DNA. This is very easily removed with a 10 second spin. The presence of these particles does not reflect a poor preparation. Once the particles are removed, the DNA performs well in all applications.

Q: Why do you recommend LB when I can get much stronger growth with other media (TB, Circle Grow, etc?)

A: The volumes of culture recommended in the kit assume an OD₆₀₀ of no more than 2.0. Rich media can be used, but the volume processed should be reduced by 1/3 to avoid overloading the column.

Q: How can I purify a DNA sample that is not in a cleared lysate?

A: Dissolve the DNA in 1 mL of 600 mM NaCl, 100 mM sodium acetate (pH 5.0). Load this onto an equilibrated column and proceed with the remainder of the protocol. Use a column appropriate to the amount of DNA to be purified. This can be used to remove RNA, proteins, and other impurities. However, genomic DNA or other plasmid forms will not be removed.

Q: What is the largest size of DNA that can be purified with these columns?

A: We have purified a 109-kb Bacterial Artificial Chromosome (BAC) with the midiprep system. We obtained ~6 ug DNA from 100 mL of culture using our recommended modified protocol. In addition, we have obtained pure Bacmid DNA (~150 kb) using the standard protocol with the PowerPrep™ HP Plasmid Miniprep kit. This DNA was used successfully in transfection of Sf9 cells. [Suggest PowerPrep HP BAC Buffer Kit?](#)

Q: Why does my plasmid have contaminating RNA?

A: There are several reasons this can occur:

- The column may have been overloaded. Verify that the culture that was used was grown to the proper density.
- Make sure that the RNase A was added and thoroughly dissolved in the Cell Suspension Buffer. It should not be stored for more than 6 months at room temperature after being dissolved.
- The time between loading the column with lysate and the first wash step might have been too long. Once the lysate is loaded onto the column, let it flow through IMMEDIATELY and then wash it immediately.
- Low copy number plasmids may have some contaminating RNA due to the large number of cells being processed. In this case, you can increase the concentration of RNase in Solution E1 from 100 µg/mL to 400 µg/mL if necessary.

Q: Why does my plasmid have genomic DNA present?

A: If contaminating genomic DNA is seen, there may have been problems with the cell lysis and neutralization steps. Perhaps mixing was too harsh or not thorough enough during the lysis and/or neutralization steps. Intact bacterial DNA forms a network during the neutralization step and is precipitated together with the SDS. This separation is only successful if no shearing of chromosomal DNA has taken place. Shearing of bacterial chromosomal DNA occurs if the sample is vortexed.

Q: Why is my Cell Lysis Solution cloudy?

A: This is common if the solution gets too cold because that causes the detergent to precipitate out. If this occurs, warm the solution briefly to 37°C until the precipitate dissolves.

Q: Why is my column not flowing properly?

A: Be sure that the column is equilibrated with Equilibration Buffer before the cleared lysate is added. After neutralization, mixing must be performed carefully, but very thoroughly (e.g. by fast inverting) until a homogeneous phase is obtained. If mixing is too vigorous, shearing of bacterial chromosomal DNA may occur. However, if the lysate has not been mixed to homogeneity, then bacterial debris and SDS may not be completely removed by centrifugation and the column will become clogged.

Q: Why do I have extra bands present in my plasmid prep?

A: This is most likely due to the presence of permanently denatured plasmid DNA that formed during the denaturation step. Be sure to incubate the lysate at RT for no longer than 5 minutes once the Lysis Solution is added.

Q: Why did my DNA degrade after restriction digestion?

A: Although the PowerPrep™ HP Plasmid kit is very good at removing all protein from the prep, this problem can very occasionally occur with low copy plasmids grown in endA+ strains. If using such strains can't be avoided, don't let the bacteria grow for more than 16 hours. Resuspend the pelleted DNA in TE buffer and then heat for 10 minutes at 70°C to inactivate any residual nucleases.

Q: Why does my DNA have a low A_{260}/A_{280} ratio (less than 1.8)?

A: If bacterial debris and SDS were not efficiently removed by centrifugation, bacterial contaminants may elute with the DNA and increase the A_{280} . Remove bacterial debris and SDS by centrifugation with at least 15,000 x g. After centrifugation to pellet SDS and bacterial debris, some particles may remain floating in the clarified lysate. Take care not to transfer these particles into the column as the contaminants associated with these particles may elute with the DNA and increase the A_{280} .

Q: Why doesn't my plasmid work in my application?

A: The plasmid may not have been alcohol precipitated, which results in a prep that has a lot of salt and relatively high pH. Follow the directions for precipitating the plasmid. For very salt-sensitive restriction enzymes, reprecipitate the plasmid and wash well with 70% ethanol.

If bacterial debris and SDS were not efficiently removed by centrifugation, bacterial contaminants may elute with the DNA and inhibit downstream applications. Remove bacterial debris and SDS by centrifugation with at least 15,000 x g.

After centrifugation to pellet SDS and bacterial debris, some particles may remain floating in the clarified lysate. Take care not to transfer these particles into the column.

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

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