Frequently Asked Questions for
Performa® Spin Columns

General

1. **<u>There is a crack in the gel matrix of the cartridge. Will it affect the cartridge performance?</u>** The crack is a normal product of the manufacturing process of the cartridges and does not affect their performance as long as the samples are loaded properly. Load samples drop-wise to the center of the matrix, taking care to avoid adding sample directly into the crack.

2. **<u>Which are the recommended spinning conditions?</u>** Spinning conditions vary depending on the volume loaded in the cartridge. For volumes lower than 20 µl, pre-spin the cartridge at 850 x g for 3 minutes, transfer the cartridge to a clean tube, load the sample onto the cartridge and spin at 850 x g for 3 minutes. For volumes equal or larger than 20 µl, pre-spin the cartridge at 750 x g for 2 minutes, transfer the cartridge to a clean tube, load the sample onto the cartridge and spin at 750 x g for 2 minutes.

Volume

3. **<u>How much volume will I get my product in?</u>** The volume of the eluted product should be approximately 5-8 µl higher than the initial sample.

4. **<u>I don't get any volume after the second spin.</u>** Check the spinning conditions and make sure that the rpm has been properly calculated. Check storage conditions and make sure that the cartridges and bag were properly closed if stored at room temperature for a short time. Cartridges should be at 4°C for long-term storage.

5. **<u>I get too much volume after the second spin</u>.** Check the spinning conditions and make sure that the rpm has been properly calculated. The presence of highly hydrophilic molecules in the reaction (such as PEG) will affect the performance of the cartridge, resulting in higher elution volume.

6. **<u>What is the maximal loading volume?</u>** For best results we recommend using volumes up to 50 µl. Larger volumes loaded onto the column may result in contaminations of small molecules (*e.g.* salts, nucleotides)

7. **<u>What is the minimal loading volume?</u>** For best results we recommend using volumes equal or larger than 10 µl. Smaller volumes loaded onto the column will result in loses and dilution of the DNA in the sample.

8. **<u>If I have a sample larger than the recommended loading volume, can I load it in several times? (load 50µl, spin, load another 50µl and so on).</u>** No. Doing so will contaminate the sample with salt and other small molecules. We recommend 50µl to achieve best results. However, 100 µl load will result in small contaminations (for example, salt removal of 5M NaCl will be of 95%) that will not affect most downstream applications. To achieve better purification at 100 µl volume loaded, we recommend to prespin 3 minutes at 750 x g, transfer the cartridge to a clean tube, load the sample, and then spin 2 minutes at 750 x g. If volumes are larger than 100 µl, we recommend splitting the sample and use two different DNA clean up cartridges.

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DNA Recovery

9. **How can I maximize the concentration of the DNA in my eluted product?** For small sample volumes (lower than 20μl) higher concentration of the sample can be achieved as follows: pre-spin the cartridge at 750 x g for 3 minutes, transfer the cartridge to a clean tube, load sample onto gel matrix and spin the cartridge at 750 x g for 2 minutes. These conditions will also result in better removal of contaminants for larger volumes (50 or 100 μl). However, these spinning conditions will result in lower recovery of total DNA (the total DNA recovered is lower, the volume is much lower, so the concentration is higher).

10. **Why didn't I recover any DNA?** Make sure that you are using the right spinning conditions. Low mass of DNA loaded will result in higher percentage losses of the DNA. Increase the total amount of DNA loaded to higher than 50 ng.

11. **The concentration of DNA in my reaction is very low. Will I lose it if I clean up the reaction through a cartridge?** If the total amount of DNA loaded is lower than 50 ng, the DNA recovery will decrease. We recommend increasing the total mass of DNA loaded.

12. **What is the oligo cut-off for the cartridges?** There is not an exact oligo cut-off in gel filtration. The percentage of oligo recovered will depend on the size of the oligo, the volume loaded on the gel, the total mass of oligo loaded and the spinning conditions.

Storage

13. **I store the cartridges in the upper part of the refrigerator close to the fan and the cartridges may have frozen. Will that be a problem?** Yes. If the gel matrix has frozen, the cartridges will not perform well. The elution volume may increase and the recovery DNA will be lower than expected.

14. **I left the cartridges over the weekend on the bench. Will that be a problem?** It will not be a problem as long as the cartridges were properly closed and the bag was sealed.

15. **Does the gel matrix contain preservatives?** No, the gel matrix is dispensed in deionized water.

Applications

16. **Do the cartridges remove detergents?** The cartridges remove SDS. Non-ionic detergents may not be efficiently removed.

17. **How much salt is removed?** It depends on the volume and the amount of salt. For most applications they will remove 99.99% NaCl and other small molecules.

18. **Can I use the cartridges to remove phenol from a phenol extraction?** 99.95% of residual levels of phenol after a phenol extraction will be removed. Cartridges cannot be used to remove pure phenol (*i.e.* do not load concentrated phenol onto the cartridge).

19. **Do the cartridges remove DMSO?** Yes.

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20. **<u>Can I use the cartridges to clean up a PCR reaction?</u>** They will remove salt, dNTPs and some of the oligo (depending on the length and mass of the oligo and the volume loaded). It will not remove DNA-polymerase or other proteins. To clean up PCR reactions we recommend Edge QuickStep® PCR clean up kit.

21. **<u>Can I use the cartridges to clean up a restriction digestion? Will it remove the enzymes?</u>** The cartridges will remove all salt and buffers. They will not remove enzymes. If enzyme removal is important, reactions should be heat inactivated prior to passing them through the cartridge (refer to enzyme manufacturer for inactivation conditions). If the enzymes cannot be heat inactivated, we recommend using Edge QuickStep® PCR clean up kit.

22. **<u>Will the cartridges remove proteins?</u>** No, most proteins will go through the gel matrix.

23. **<u>Can I use the cartridges to remove PEG from a ligation?</u>** PEG and other highly hydrophilic molecules will cause the cartridges operate inefficiently. The elution volumes will increase and the DNA recovered will decrease.

24. **<u>Can I use the cartridges to clean up miniprep DNA prior to mammalian transfection?</u>** It depends on how clean the DNA is and how sensitive the mammalian cells are. We have achieved higher than 80% transfection efficiency in 293 HEK cells using miniprep DNA passed through Edge DNA clean up cartridges. The cartridges will remove salt and other small molecules, but they will not remove all endotoxins.

25. **<u>Are the cartridges RNase free?</u>** We cannot guarantee that the cartridges are RNase free, since we do not test for it in Quality Control. However, we operate in clean hoods and our manufacturing facilities have clean air filters, lowering the probabilities of RNase contamination in the cartridges. Some of our customers are using the DNA clean up cartridges for RNA work and they have not experienced any problems.

26. **<u>Are the cartridges DNase free?</u>** Yes, we test for DNase contamination in the Quality Control of the DNA clean up cartridges.

27. **<u>Are the cartridges sterile?</u>** We cannot sterilize the cartridges after filling them and we do not include preservatives in the gel matrix. However, we use sterile water to swollen and wash the gel matrix; we operate in clean hoods and our manufacturing facilities have clean air filters. We test a sample of our cartridges for contamination as part of the Quality Control.

28. **<u>Will the product be eluted in water?</u>** We only use sterile deionize water in all steps during swelling, washing and final suspension of the gel matrix. However, due to the manufacturing process of the resin that we use in the gel matrix, a small amount of salt (approximately 3 mM) leaches from the gel and is present in the final elution. This amount of salt does not affect any standard downstream application.

29. **<u>Will the eluted product be denatured?</u>** If using very short DNA samples, salt should be added to the eluted product. The salt concentration should vary depending on the downstream applications; for most applications we suggest using a final concentration of 10 mM Tris (pH 7.5-8), 5 mM NaCl, 0.1 mM EDTA. We also recommend not heating short DNA samples before adjusting salt concentration to avoid denaturing of the sample.