



Frequently Asked Questions for Performa® DTR 384 Well Plates

Sample Recovery

1. **What is the minimum sample volume?** The recommended minimum volume is 5 μL . Smaller volumes loaded onto the column will result in low signal strength, loss of 5' sequence, and shorter read lengths.
2. **What will be the final volume of the purified sample?** The final volume of the purified sample should be approximately 1-4 μL higher than that of the initial sample.
3. **Why is the volume of my purified sample greater than 4 μL more than the volume loaded?**
 - (a) Your plate may have been frozen. Freezing and thawing usually results in higher than normal recovery volumes. Freezing will also negatively affect the performance of this product.
 - (b) Centrifugation conditions are essential to the optimum performance of this product. If the centrifuge is new or different, or you are a first-time user of the product, be aware that not all centrifuges behave the same to any given set of instructions. Acceleration and deceleration rates are often different and this may affect your results. First, check the spin conditions and make sure that the rpm has been calculated correctly and the spin times are correct. If the calculations are correct, then consider that centrifuges with slow acceleration rates are particularly susceptible to this type of problem. Try extending the length of the initial and final spin by 30 seconds.
4. **Why is my purified sample volume less than my original load volume?**
 - (a) The plate may be dehydrated. To avoid this, plates should be stored at 4° C in their original packaging.
 - (b) Centrifugation conditions are essential to the optimum performance of this product. If the centrifuge is new or different, or you are a first-time user of the product, be aware that not all centrifuges behave the same to any given set of instructions. Acceleration and deceleration rates are often different and this may affect your results. First, check the spin conditions and make sure that the rpm has been calculated correctly and the spin times are correct. If the calculations are correct, then consider that centrifuges with fast acceleration rates are particularly susceptible to this type of problem. Try decreasing the length of the initial spin by 30-60 seconds.
5. **I centrifuged the plate at a speed much lower than recommended. Can I still recover my sample?** Spin the plate for an additional minute at the correct g force. Even though this procedure may "save" your sample, it will not be optimal. It will likely have low signal strength and may be contaminated with unincorporated dye terminators. You may need to repeat the sequencing reaction.

General

1. **What are the recommended spin conditions?**
 - (a) **For 5 μL Reaction Volumes:** Pre-spin the plate at 850 x g for 2 minutes, load the samples onto the plate and spin at 850 x g for 5 minutes.
 - (b) **For 10 μL Reaction Volumes:** Pre-spin the plate at 850 x g for 2 minutes, load the samples onto the plate and spin at 850 x g for 2 minutes.



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- My centrifuge starts timing before the rotor has reached full speed, will this affect the time settings I should use?** The recommended protocol assumes that timing starts when the centrifuge starts spinning. Acceleration and deceleration times are highly variable and on some centrifuges they are controllable and on others, they are not. The recommended protocol is an approximation of the best conditions for using this product. It may be necessary to adjust the spin conditions slightly from the recommended protocol to achieve optimum results.
- Why does the chromatogram show dye blobs?** Unincorporated dye terminators co-migrate with sequencing products during electrophoresis. The most common peaks occur roughly 70 bases into the sequencing run, and in cases of severe contamination additional peaks may appear in the vicinity of base 120, 220, or higher. Unincorporated dye terminators can appear in purified products for a variety of reasons.
 - Unincorporated dye terminators will co-migrate with the sequencing products if the sample is allowed to pass down the side of the well rather than through the gel matrix; always use care when applying the sample drop-wise to the center of the gel matrix.
 - The plates may have been frozen. Freezing irreversibly damages the integrity of the gel matrix and samples processed on these plates will fail. Centrifugation conditions are essential to the optimum performance of this product.
 - Make sure that the rpm has been calculated correctly and that the spin times are correctly entered. If conditions are correct, it may be necessary to optimize centrifugation protocol for your specific centrifuge, following recommendations described in answers to 4b and 5b of the **Sample Recovery** section.
- How do I measure the radius?** The radius is equal to the distance in millimeters between the axis of rotation and the bottom of the gel bed when the plate and the receiver are placed in the plate carrier in the centrifuge bucket.

To achieve RCF = 850 x g:

$$rpm = \frac{27,549}{\sqrt{r}}$$

- Can I use BigDye® v. 3.1?** Yes. For best results use no more than 0.5X BigDye® v 3.1 concentrations, e.g. 2 µL of BigDye® v 3.1 per 10µL reaction.

Storage

- I realized the plates are frozen. Will that be a problem?** Frozen plates cannot be used. The matrix ruptures and seals the base of the unit resulting in total sample loss.
- Can I store plates at room temperature for short periods of time?** Yes. A few hours on the bench or overnight storage will not damage performance or reduce shelf life as long as the plates are stored in their original packaging. We do not recommend long-term storage at room temperature.
- Does the gel matrix contain preservatives?** No, the gel matrix is dispensed in deionized water.