



Frequently Asked Questions for MagDTR™ Technology

Resin Suspension

1. **I'm having trouble with the beads settling. How can I keep them in uniform suspension while dispensing into the samples?** Shake well or vortex the resin container until the resin is in complete suspension. For all subsequent pipetting steps, use a pipet to mix the suspension prior to dispensing into the sample.
2. **Can you suggest any other form of mixing besides pipet mixing?** If using a multichannel pipet reservoir, resin can be resuspended by rocking the reservoir back and forth 7-10 times.

Mixing

1. **Is the number of wash step pipet mixes flexible?** No. A minimum of 15 pipet mixes gives consistent purity and yields.
2. **Can I replace pipet mixing with vortexing throughout the protocol?** Vortex mixing during the protocol process is not recommended.

Precipitation

1. **Can I bind the sequencing reaction products to the magnetic resin with solvents other than ethanol?** Other solvents, especially isopropanol, are not recommended.
2. **Can I use 80% alcohol to bind the sample to the resin?** Yes. However, 5 volumes of 80% ethanol are required to bind the sequencing reaction products. This large increase in total solution volume may obviate the advantages of using a single alcohol concentration for all steps.
3. **Can I premix the resin with ethanol prior to purification?** No. Premixing the MagDTR resin with ethanol negatively impacts the performance of the product.
4. **Can I reduce the amount of resin for smaller volume reactions and/or for lower amounts of BigDye®?** The optimum amount of resin to use is 4 ul, and it has been our observation that this volume is the optimum over a broad range of conditions.
5. **Can I use this technology with other dyes other than BigDye®?** Yes, including Beckman DTCS™ and Amersham dyes.



Frequently Asked Questions for MagDTR™ Technology

Washing

1. **Is there any advantage to washing more than once** While one wash may be sufficient for very low dye volumes, two washes give far more reproducible results over a range of dye volumes up to 4 μ l. A third wash step can increase the consistency of results when using higher dye volumes.
2. **Can I use different concentrations of ethanol for the wash step?** The optimum concentration of ethanol is 80%. Concentrations as low as 60% and as high as 90% have been tested. These other concentrations are reasonably effective at washing the resin. Lower concentrations of ethanol tend to reduce signal strength and read length especially for samples containing small amounts of DNA. Increased ethanol concentrations are less effective at washing away salts and unincorporated dye terminators leading to an increased frequency of dye blob contamination, noise, and reduced signal strength.
3. **Does residual ethanol affect the quality of the sequence?** Yes. In most cases, residual ethanol from the wash steps will contain trace amounts of unincorporated dye terminators. Residual ethanol must be removed to obtain optimal results.
4. **I am seeing “dye blobs.” What is the cause?** Blobs or unincorporated dye terminator peaks are usually a result of incomplete removal of ethanol that may contain unincorporated dye terminators. If residual ethanol is present following the first aspiration, a second aspiration is recommended. Offset the pipet to 150 μ l, and aspirate as slowly as possible to remove all residual ethanol. If this step alone does not solve the problem, then try adding a third wash step to the process.
5. **I can't seem to get rid of residual ethanol. Any suggestions?** Add an extra aspiration step after each ethanol removal, aspirating as slowly as possible with the pipet offset to 150 μ l. If this doesn't solve the problem, then try adding a third wash step.
6. **Can I over dry the resin?** Yes. Over dried resin will be attached to the plate well wall in an obvious manner and will be difficult to work with during resuspension. Over drying of the resin may result in reduced signal strength and an increase in the overall failure rate. To avoid over drying, aspirate all ethanol, and reduce drying time at room temperature to 3 minutes, or heat at 98°C for 30 seconds. Resuspend immediately. If samples appear over dried at the elution step, add the recommended volume of deionized water, and incubate at room temperature for one minute. Pipet mix to resuspend.
7. **How can I tell when the resin is optimally dry?** Resin is optimally dry when there is no visual evidence of residual ethanol following the drying step.



Frequently Asked Questions for MagDTR™ Technology

Elution

1. **Can I elute my sample in formamide?** No. However, we recommend that users of this sample purification system follow the instrument manufacturer's recommendation for the appropriate solvent to be used in sample injection. If the sample must be in formamide, the sample can be eluted in water then dried down and resuspended in formamide.
2. **Can I load my plate into the sequencer with the resin present?** This is not generally recommended since the presence of particulates in the sample carries the risk of clogging the capillaries in the DNA analyzers.

Storage

1. **Can I store the sample before eluting from the resin?** Yes, but only after completing the wash steps. The samples should be sealed to prevent over-drying and stored at 4°C. This has been tested for up to one week storage. At this time we do not recommend storage for longer periods.
2. **How do I store the sample after it has been eluted from the resin?** Remove the sample from the resin, and store at - 20°C.
3. **Can I store the sample with resin after it has been eluted?** No. We do not recommend this procedure.
4. **Can I store the MagDTR resin in the refrigerator?** The product may be stored at 4°C, but be sure to bring the resin to room temperature prior to use. Avoid freezing the product.

Magnet

1. **What kind of magnet is recommended?** We recommend the **Edge BioSystems' MagWell™ Magnetic Separator 96, Catalog #57624**. The unique configuration of the magnet (milled cavities aligned on either side of an imbedded bar magnet) places the tip of the wells below the top of the bar magnets on the plate. This configuration maximizes the magnetic field strength experienced by each well while allowing the magnetic particles to be pulled both to the side and away from the bottom of the plate. In so doing, it permits effective washing of the particles in both magnetic and manual liquid handling protocols. It also permits the user to resuspend in a minimum volume of fluid e.g. 10 µl. If using an alternative magnet, the protocol will need to be optimized by the user.