Product Information

Diffinity RapidTip® 50
for PCR Cleanup

Technical Bulletin

Product Description

The Diffinity RapidTip® 50 contains everything needed for PCR cleanup of a 50 µl reaction (see Procedure). The functionalized Tip contains a proprietary adsorption resin, which has a differential affinity for PCR reaction components. The impurities are removed from the solution as it enters the pipette tip and, after mixing for one minute, dispensing the solution yields high-quality DNA ready to use for downstream applications. This RapidTip® product is a 200 µl pipet Tip compatible with most universal pipettors.

Product Use

Diffinity RapidTip® 50 is designed for cleanup of PCR solutions prior to dideoxy (BigDye®) reaction for Sanger Sequencing. Diffinity RapidTip® 50 effectively removes up to 90% of dNTPs, primers, and primer-dimers while providing up to 90% recovery of pure DNA fragments ranging in length from 100 bp to 10 kb. RapidTip® 50 is not compatible with detergents, mineral oil, or ready-to-load style PCR master mixes that contain a density increasing compound.

Reagents Supplied

Material	Catalog Number	Quantity
Diffinity RapidTip® 50 for PCR Cleanup	RT050-096	96 RXN

Equipment and Reagents Required But Not Provided

- PCR Sample
- Tubes to store DNA
- Pipettor – manual or programmable; single channel or multichannel
- Standard pipette tips for liquid transfer (if needed)

Precautions and Disclaimer

Diffinity RapidTip® 50 is for R&D use only, not for drug, household, or other uses. Consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Guidelines for Pipetting with the RapidTip

Careful pipetting is important to achieve effective clean-up with the RapidTip®. The following pipetting guidelines will help ensure optimal results:

1. Preparing the Tips

   Before aspirating any sample, tap the box of Tips on the benchtop to ensure all of the resin is off of the sides of the Tip and in the mixing area. If this isn't sufficient, the pipettor can initially be set to a higher volume (150-170 µl) for the first aspiration to allow the sample to collect the resin as it travels upwards. After this first aspiration, fully dispense, and reset the pipettor to the appropriate volume (see Procedure).

2. Pre-wet the Resin
   On the first aspiration step, allow a 5 second pause before dispensing to ensure complete wetting of the resin. After this first pause, continue pipetting at a rate of roughly 1 cycle per 2 seconds.

3. Maintaining resin-to-sample contact

   It is important to make sure the sample is always in contact with the resin in the Tip. To this end, it is helpful to not fully dispense the sample after each cycle. This ensures constant contact of the resin to the sample, and helps prevent detachment.

4. Recovering from resin detachment

   Should the resin become detached from the sample (this will look like a small "bubble" that moves independent of the sample), gently "flick" the pipettor downward to bring it back into contact with the sample and proceed with mixing. Additional time for treatment might be need to be added.

Storage/Stability

Store the tips at room temperature

Procedure

Prepare Samples:

Diffinity RapidTip® 50 is optimized for 50 µl reaction volumes, but can effectively purify samples from 45-55 µl.

- For PCR volumes >55 µl, aliquot 50 µl into each tube for purification.
- For PCR volumes <45 µl, dilute to 50 µl using 1X PCR buffer. This works best with highly concentrated DNA samples (>50 ng/µl) as your sample concentration will be reduced.

Prepare Tips:

Diffinity RapidTip® 50 contains proprietary resin that cleanup a PCR reaction; this resin can adhere to the pipette tip walls during shipping. For optimal results, sharply tap the box 2-3 times on a flat surface so that the resin is near the bottom of the tips (near the retainer).

Purify Samples:

1. Set or program the pipettor to aspirate 75 µl (unless performing #1 in the Guidelines for Pipetting section, in which case set the pipettor to 150-170 µl).
2. Place Diffinity RapidTip® 50 on the pipettor.
3. Place pipette tip into the 50 µl PCR solution.
4. Pre-wet the resin on the first aspiration (see Pipetting Guidelines).

*If you've chosen to perform #1 in the Guidelines for Pipetting section, at this point, fully dispense the solution, reset the pipettors to 75 µl, and proceed with step 5 below.

5. Mix for 60 seconds (approximately 30 aspirate/dispense cycles).
6. Dispense all solution into a clean tube when mixing is complete. Use your pipettor's blowout mode for maximum liquid recovery.

The cleaned PCR amplicon is now ready for downstream Sanger sequencing. If you wish to confirm purification, run unpurified and purified samples in adjacent lanes on a gel to confirm the amplicon bands. To estimate percent recovery, analyses of the samples pre- and post-cleanup are necessary. For this process, we recommend either a PicoGreen®2 type assay or visualization on an agarose gel.

T**roubleshooting Guide**

Problem	Solution
Low DNA Recovery	An optimization of the treatment time might be needed. While one minute is usually sufficient for most samples, occasionally less or more time might be needed to achieve optimal results.
	Ensure the concentration of the starting solution isn't too low. It's generally recommended that the starting DNA concentration be around 50 ng/µl.
Slow or difficult aspiration	Check that the pipettor volume is set to 75 µl.
	Check that the Tips are firmly attached to the pipettor. Note: Diffinity RapidTip® 50 is incompatible with detergents, mineral oil, and ready load PCR mixes that contain density increasing compounds.
Fluid remains in the RapidTip®	It is normal for a small amount of liquid (~5 µl) to remain inside the Diffinity RapidTip®. Note: Over-dispense or blow all fluid out on the last dispense cycle.
Failure to remove impurities	Verify that the resin is at the bottom of the Tip prior to sample treatment.
	Ensure the resin is effectively mixing with the sample during pipetting.
	Ensure the sample isn't too concentrated. It's generally recommended that the starting DNA concentration be around 50 ng/µl.