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Product Information

Diffinity RapidTip®2
for PCR purification with Polymerase Removal

Catalog Number **D2947**

TECHNICAL BULLETIN

Product Description
The Diffinity RapidTip2 contains everything needed for PCR purification with polymerase removal. The functional tip contains a proprietary adsorption technology 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 just one minute, dispensing the solution yields purified, high quality DNA ready to use for downstream applications.

Product Use
Diffinity RapidTip2 is designed for purification of PCR solutions prior to dideoxy (BigDye®) reaction for Sanger Sequencing, restriction digest, and T-A cloning. The RapidTip2 is compatible with most universal pipettors. Diffinity RapidTip2 effectively removes DNA polymerase, in addition to up to 90% of dNTPs, primers, and primer dimmers, while providing up to 90% recovery of pure DNA fragments ranging in length from 100 bp to 10 Kb.The RapidTip2 is optimized for a 50 µL PCR reaction sample.

Reagents Supplied

Material	Catalog Number	Quantity
Diffinity RapidTip2 for PCR Purification with Polymerase Removal	D2947	8 RXN
		48 RXN
		96 RXN

Equipment and Reagents Required But Not Provided
- PCR Sample
- Tubes to store purified DNA
- Pipettor- manual or programmable
- Standard pipette tips for liquid transfer (if needed)

Precautions and Disclaimer
The Diffinity RapidTip2 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 RapidTip2
Careful pipetting is important to achieve effective purification with the RapidTip2. The following pipetting guidelines will help ensure optimal results.

1. Pre-wet the particles
   On the first aspiration step, aspirate approximately the first half of the sample and pause 5 seconds to ensure complete wetting of the particles before mixing. Then aspirate the rest of the sample and dispense.
2. Mix Effectively
   For effective mixing, it is important that the particles remain in contact with the sample during each aspirate and dispense cycle.

   Aspirate slowly for the first several cycles until the particles are completely dispersed into the PCR sample.

   Avoid long delays between successive aspirate and dispense cycles that allow the particles to settle out of solution for more than 1-2 seconds.
3. Maintain tip-to-sample contact
   During aspiration, maintain continuous contact between the end of the pipette tip and your sample to avoid aspirating air bubbles in the sample.
4. Maintain tip-to-well clearance
   Maintain clearance between the end of the pipette tip and the bottom of the sample tube or well to avoid plugging the tip during sample mixing.
5. Recovering from particle detachment
   If particles become detached from the sample, gently “flick” the pipettor downward to bring them back into contact with the sample and proceed with mixing.

Storage/Stability
Store the tips at room temperature.

Procedure

Prepare Samples:
Diffinity RapidTip2 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. This works best with highly concentrated DNA samples (> 50 ng/ µL) as your sample concentration will be reduced.

Prepare tips:
Diffinity RapidTip2 contains proprietary particles that purify a PCR reaction. These particles 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 particles are at the bottom of the tips (near the retainer).

Please note that it is normal to see fine dust-like particles on the side of the tip. After tapping the box, expect to see about 3 mm of white particles above the retainer at the small end of the pipette tip.

Purify Samples:
1. Program pipettor to aspirate 60 µL
2. Place Diffinity RapidTip2 on pipettor.
   Note: A multi-channel pipettor can be used to mix more than 1 sample at a time for even higher productivity.
3. Place pipette tip into 50 µL PCR solution.
4. Pre-wet the particles on first aspiration (see Pipetting Guidelines)
5. Mix for 60 seconds (approximately 15 aspirate / dispense cycles).
   Note: Pipetting will be slower than normal. Wait for liquid to completely fill the tip to begin the next mix. It is not necessary to drive liquid completely out of the tip on every dispense. The particles should mix completely with the solution and make it appear cloudy.
6. Dispense all solution into a clean tube when mixing is complete. Use your pipettor's blowout mode for maximum liquid recovery.

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

BigDye is a registered trademark of Applera Corp.
PicoGreen is a registered trademark of Molecular Probes, Inc.
RapidTip2 is a registered trademark of Diffinity Genomics, Inc.

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Troubleshooting Guide

Problem	Solution
Low DNA Recovery	Run some of the untreated and RapidTip2 purified PCR on a gel to check amplicon band intensity. **Note:** Purify at lease 45 µL of the PCR reaction to optimize DNA recovery.
Slow or difficult aspiration	Check that the pipettor volume is 50 µL
	Check that the tips are firmly attached to the pipettor. **Note:** Diffinity RapidTip2 is incompatible with detergents, mineral oil, and ready load PCR mixes that contain density increasing compounds.
Fluid remains in the RapidTip2	It is normal for a small amount of liquid (~5 µL) to remain inside the Diffinity RapidTip2. **Note:** Over-dispense or blow out all fluid on the last dispense cycle.
Failure to remove impurities	Verify that particles are at the bottom of the tip prior to sample treatment; the pipettor is operating at the proper (slow) speed settings; and that particles are effectively mixing with the sample during pipetting. **Note:** Remove trapped air bubbles by tapping the tip or mix for additional aspirate/dispense cycles.