

USER HANDBOOK

Version G (August 2012)

DIFFINITY RapidTip® For PCR Purification

Important User Notice:

For optimal purification results, sharply tap the RapidTip package 2–3 times on a flat surface so that the particles drop to the bottom of the tips.

Diffinity
Genomics™

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Kit Contents

Diffinity RapidTips can be used with universal or Rainin LTS® style pipettes. All kits come with a user handbook.

Cat Number	Product Description	Contents
RT025-008 RT025-008L*	Diffinity RapidTip for PCR Purification	1 Pack of 8 RapidTips
RT025-048 RT025-048L*	Diffinity RapidTip for PCR Purification	1 Box of 48 RapidTips
RT025-096 RT025-096L*	Diffinity RapidTip for PCR Purification	1 Box of 96 RapidTips

* L — denotes tips compatible with Rainin LTS® pipettes

Storage

Diffinity RapidTips should be stored in low humidity at room temperature (15–25 degrees Celsius). Under these conditions, Diffinity RapidTips can be stored and used for up to 12 months without reduction in performance or quality. An expiration date is on the label of the 48 and 96 tip boxes.

Product Use Limitations

Diffinity RapidTips are intended for research use only. Diffinity RapidTip is designed for purification of PCR solutions prior to dideoxy (BigDye) reaction for Sanger Sequencing. RapidTip is compatible with most universal pipettors. RapidTip is not compatible with detergents, mineral oil, or ready-to-load style PCR master mixes that contain a density increasing compound.

Product Warranty and Satisfaction Guarantee

Diffinity Genomics will guarantee the performance of Diffinity RapidTips for the uses and performance as described in our product literature. If the product fails to perform to your satisfaction for any reason other than misuse, we will replace the product free of charge or refund the purchase price — whichever you choose. Diffinity Genomics maintains the right to update or modify the product to enhance its performance. A copy of our terms and conditions can be found on our web site.

If you have questions about the product or its performance, please contact Diffinity Genomics customer service at 1-877-362-1812.

Quality Control

Each lot of Diffinity RapidTips is tested against predetermined specifications to ensure consistent quality.

Technical Assistance

Call 1-877-362-1812 between 8am and 4pm Eastern Time Monday through Friday or visit www.diffinitygenomics.com for help with common questions.

Safety Information (Disposal)

Diffinity RapidTips may be disposed in regular lab trash. For more information, please consult the material safety data sheet (MSDS) available on request by calling Customer Service at 1-877-362-1812.

Product Specifications

Capacity: PCR Sample Size	25 ±5 microliters
dsDNA Recovery	up to 90% (100bp to 10Kb)
Impurity Removal (dNTP, primers, primer-dimers)	up to 90% (less than 45–50 bp)

*Please note that this Diffinity RapidTip for PCR Purification does not remove DNA polymerase. For applications requiring the removal of DNA Polymerase, please use the **Diffinity RapidTip2** for PCR purification with Polymerase Removal.*

Standard DNA polymerase such as Taq does not typically interfere with the sequencing amplification (BigDye) reaction.

Introduction

Diffinity Genomics has developed an innovative new technology that removes undesirable impurities from PCR reactions leaving you with nothing but purified DNA!

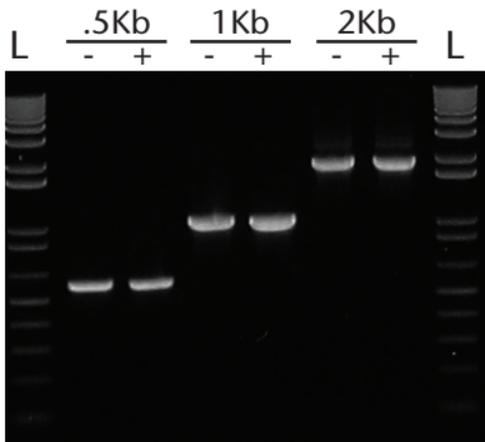
This revolutionary technology effectively removes dNTPs, primers, and primer-dimers while providing up to 90% recovery of pure DNA fragments ranging in length from 100bp to 10Kb. It dramatically reduces the amount of time to purify and prepare PCR solutions prior to dideoxy (BigDye) reaction for Sanger Sequencing.

The RapidTip for PCR purification requires just one-tip, one-step, and one-minute for PCR purification!

Diffinity™ Technology Principle

The Diffinity RapidTip contains everything you need for PCR purification prior to Sanger sequencing. The tip is filled with our 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.

Dispensing the solution yields purified, high quality DNA ready to use for downstream applications. You won't need additional equipment, reagents, buffers, or enzymes — only a pipettor!



Diffinity RapidTip Purification has excellent yield. Equal volumes of untreated (-) and Diffinity RapidTip treated (+) PCR product (.5Kb, 1Kb, 2Kb) were run on a 1% agarose gel alongside Invitrogen 1Kb Plus Ladder (L) and labeled with SybrSafe.

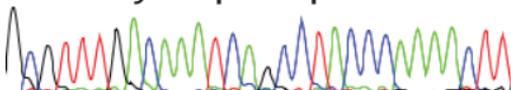
GC G T T T G A C A A A T C A G C C T A C C C A A A A C T T
500 510 520

unpurified



GC G T T T G A C A A A T C A G C C T A C C C A A A A C T T
500 510 520

Diffinity RapidTip



Diffinity RapidTip purification improves sequence quality. Sequence chromatograms show improved peak quality and lower background in Diffinity RapidTip purified samples as compared to unpurified samples.

Equipment to be Supplied by User

- PCR Sample (25 μ l \pm 5 μ l)
- Tubes to store purified DNA
- Pipettor — P100/P200 manual or programmable
- Standard pipette tips for liquid transfer (if necessary)

Guidelines — Pipetting with the RapidTip

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

1. Pre-wet the particles

On the first aspirate 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. Aspirate slowly

For effective mixing, it is important to aspirate slowly with each successive cycle to ensure that particles remain in contact with the sample.

If using a **manual pipettor**, aspirate slowly for the first several cycles until the particles are completely dispersed into the PCR sample.

If using an **automated pipettor**, set to aspirate on low speed (2 of 10 or equivalent), dispense on middle speed (5 of 10 or equivalent) and place into manual mix mode.

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 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.

Pipetting Examples



A. Correct mixing:
Sample and particles
mixing well.



B. Incorrect mixing:
Particles adhering to
pipette tip.



C. Incorrect mixing:
Particles separated by
air bubble.

Please note that typical PCR samples are colorless — for visualization, yellow dye was used.

To correct **B** and **C** and to improve performance, gently “flick” the pipettor downward to bring all particles and the sample back together, then proceed with mixing.

RapidTip PCR Purification Protocols

Prepare Samples:

Diffinity RapidTip is optimized for 25 μl reaction volumes but can effectively purify samples from 20–30 μl .

- For PCR volumes $>30 \mu\text{l}$, please aliquot 25 μl into a new tube for purification.
- For PCR volumes $<20 \mu\text{l}$, please dilute to 25 μl . This works best with highly concentrated DNA samples ($>50 \text{ ng}/\mu\text{l}$) as your sample concentration will be reduced.

Prepare Tips:

Diffinity RapidTip for PCR Purification 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, you should expect to see about 2mm of white particles above the retainer at the small end of the pipette tip.

Purify Samples:

1. Program pipettor to aspirate 50 μl .
2. Place Diffinity RapidTip on pipettor — single or multichannel.

Please note that you can use a multi-channel pipettor to mix more than 1 sample at a time for even higher productivity.

3. Place pipette tip into 25 μ l PCR solution.
4. Pre-wet the particles on first aspirate (see page 10).

Aspirate about half the sample and then pause for 5 seconds to ensure complete wetting of the particles before mixing. Aspirate the rest of the sample and then dispense.

5. Set timer and mix for 60 seconds (approximately 15 aspirate/dispense cycles).

Please note that 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 while inside the tip.

6. Dispense all solution into a clean tube when mixing is complete. Use your pipettor's blowout mode for maximum liquid recovery.

Your purified PCR Amplicon is now ready for downstream Sanger amplification.

Quantifying DNA Recovery

If you wish to confirm purification, run unpurified and purified sample in adjacent lanes on a gel to confirm the amplicon 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 agarose gel.

Troubleshooting Guide

Problem	Things to try:
Low DNA recovery	<p>Run some of the untreated and RapidTip purified PCR on a gel to check your amplicon band intensity.</p> <p><i>Note: Purify at least 20 μl of your PCR reaction to optimize DNA recovery.</i></p>
Slow or difficult aspiration	<p>Check that pipettor volume is 50 μl. Check that tips are firmly attached to the pipettor.</p> <p><i>Note: Diffinity RapidTip is incompatible with detergents, mineral oil, and ready-to-load PCR mixes that contain density increasing compounds.</i></p>
Fluid remains in the RapidTip	<p>It's normal for a small amount of liquid (~5 μl) to remain inside the Diffinity RapidTip.</p> <p><i>Note: Overdispense or blowout all fluid on the last dispense cycle.</i></p>
Failure to remove impurities	<p>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.</p> <p><i>Note: Remove trapped air bubbles by gently "flicking" the pipettor downward for additional aspirate/dispense cycles.</i></p>

Visit www.diffinitygenomics.com/FAQs.asp for additional information.

Ordering Information

You can place an order by:

1. Contacting one of our distributors worldwide. For a complete list, visit our website.
2. Call Diffinity Genomics Customer Service and place the order by phone: **+1-877-362-1812**
3. Visit our website and place an order online via the online Diffinity store: **www.diffinitygenomics.com/order.asp**

For more information, please visit our website:

www.diffinitygenomics.com

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