Diffinity RapidTip® for PCR Purification (Pre-Sanger Sequencing)

Frequently Asked Questions

1. **Q:** What pipettors are compatible with the Diffinity RapidTip?

   A: The RapidTip currently uses a 'universal' tip that best fits Benchmate, Biohit, Eppendorf, Oxford, and Pipetman standard pipettors. We have also had customers use Alphapette, Finnpipette, Gilson/Genemate, Labnet, and Rainin traditional pipettors with successful results.

2. **Q:** What PCR sample volume can be purified with a single tip? Is there any sample loss?

   A: The RapidTip is currently optimized to purify 25 microliter PCR samples and can purify 20-30 microliters with good results. If your sample is less than 20 microliters and at a higher DNA concentration, you may be able to dilute to 25 microliters prior to purification with good results. Typically up to 4-5 microliters of volume can remain inside the tip.

3. **Q:** Do all 96 RapidTips need to be used at the same time?

   A: No, you do not need to use all 96 RapidTips at the same time; you can use as many as you need and store the remainder in the box on your bench or shelf at room temperature. RapidTip packaging was designed to be flexible since many researchers purify different numbers of samples.

4. **Q:** What is the shelf life of RapidTips?

   A: RapidTips have a shelf life of 1 year.

5. **Q:** Does the RapidTip purify PCR prior to TOPO cloning?

   A: TOPO cloning typically requires removal of primers and primer-dimers from a PCR reaction. The RapidTip removes nucleotides, primers, and primer-dimers (DNA smaller than 45-50 bp) so it should work well for TOPO cloning.

6. **Q:** Where in a typical PCR purification pipeline is the RapidTip used?

   A: The RapidTip is used for post-PCR purification – it can replace the typical spin column, enzymatic, or magnetic bead kits that are used for PCR purification. The RapidTip removes DNA impurities (nucleotides, primers, and primer-dimers < 45-50 bp) from the PCR reaction while leaving the PCR amplicon (>100bp), low amount of salt, and the polymerase in solution (for this initial product). The low of level of salt and polymerase do not interfere with the downstream

Sanger sequencing reaction in our extensive tests (salt and enzymes are also present in Exo-SAP treated PCR).

Below find an outline of a typical workflow. Step 3 (italics) is the correct place to use RapidTip.

1. DNA Extraction: Extract from tails, tissue, bacteria, etc. Purification may or may not be included in all protocols.
2. PCR amplification: Use genomic, plasmid, or other template DNA from (1) to amplify the region of interest.
3. *RapidTip Purification**: Use the Diffinity RapidTip to remove dNTP, primer, and primer-dimer from your PCR reaction – the PCR amplicon will remain in solution.*
4. BigDye Reaction: Use RapidTip purified PCR amplicon from (3.) as template for your sequencing reaction. Note: The RapidTip is not optimized to purify the BigDye reaction.

7. Q: Can the RapidTip be used to purify PCR products 50-99 bp long?

A: The RapidTip will purify 50-99 bp PCR products but with the lower yields outlined below:

- 50-75 bp: ~50% yield/recovery of dsDNA
- 75-99 bp: ~65% yield/recovery of dsDNA

Because the RapidTip removes significant amounts of dsDNA between 50 and 99 bp, the amount recovered may not be sufficient for all downstream applications – it will depend on the initial or starting DNA concentration as well as the amount of DNA necessary for downstream applications (which varies between researchers).

8. Q: Does the RapidTip pass through large single-stranded DNA (100-200 bp) such as probes for *in situ* hybridization?

A: The RapidTip has not been validated for use to recover large single-stranded DNA, however large single-strands typically self-hybridize at room temperature, which could form structures similar to dsDNA between 50 and 99 bp. If that occurs, the yield may be lower as outlined below:

- 50-75 bp: ~50% yield/recovery of dsDNA
- 75-99 bp: ~65% yield/recovery of dsDNA

Individual researchers will need to determine if recovery is sufficient for downstream reactions.

9. Q: Has the RapidTip been used in library preparation for next or second generation sequencing (SOLiD, 454, Solexa, etc?)

A: At this time, the RapidTip has not been validated for use for next or second generation sequencing library preparation. The RapidTip has been validated for use to purify PCR samples prior to Sanger sequencing.

10. Q: What are some of the benefits of the Diffinity RapidTip product?

A: The RapidTip provides a dramatic reduction in purification speed and ease of use while providing excellent yield and purity. Other benefits of the RapidTip include:

- Extremely rapid purification – only one minute (purify 8 or 12 reactions in one minute by using a multichannel pipettor)
- Removes up to 90% of the impurities (dNTPs, primers, primer-dimers)
- Recovers up to 90% of dsDNA
- Minimal labor (simple, one-step protocol)
- Easy to learn (pipetting is ubiquitous and doesn't require extensive training of existing and new personnel)
- Handles large range of fragment length – 100 bp to 10 kb
- No special storage conditions (room temperature storage - doesn't require refrigeration or freezer space)
- Small storage footprint (RapidTip comes in a standard pipette tip box)
- No special handling (no reagents, just pipette to mix)
- No special or capital equipment (beyond a simple pipettor)
- Minimal waste (only the tip is disposed after use)
- Labor cost savings - improved productivity

11. Q: Is the RapidTip compatible with PCR mastermixes and supermixes?

A: The RapidTip has not been validated for use with all pre-mixed PCR master or supermixes and we cannot guarantee that performance with a specific master or supermix will be the same as with standard PCR components. Tests have shown that detergents (surfactants) and dyes can cause bubbling and additional volume loss without a loss in purification performance.

12. Q: Is the RapidTip re-usable?

A: No, the RapidTip is a single-use purification product and is optimized to purify a single 25 microliter (+/- 5 microliter) PCR reaction.

13. Q: Does the RapidTip use a matrix/column to purify PCR product?

A: No, the RapidTip contains loose particles that are designed to mix with the solution for optimal purification. Mixing is achieved through aspiration of the sample with a standard pipettor.

14. Q: Is RapidTip appropriate for purification after multiplex preamplification reactions before analyzing samples in chips, e.g. for gene-expression. What primer concentration will RapidTip purify?

A: The RapidTip has been designed to remove impurities from a typical PCR reaction which would be < 1 microMolar primers. However, RapidTip is tested for purification of up to 3 microMolar primers and obtains successful results.

15. Q: Will RapidTip remove fluorescently labeled dNTPs from PCR solution?

A: Yes, fluorescence should not affect purification. However, please note that RapidTip does not remove salts present in the PCR buffer so RapidTip purification may not be appropriate prior to capillary electrophoresis (see question 17). Please examine purification requirements for each specific application in order to determine if RapidTip is appropriate.

16. Q: Is the RapidTip appropriate for purification prior to capillary electrophoresis?

A: The RapidTip was designed for PCR purification prior to Sanger sequencing and removes DNA impurities (dNTP, primer, primer-dimer) smaller than 45-50bp while retaining the double-stranded PCR amplicon larger than 100bp in solution. At this time, the RapidTip does not remove salt (and the RapidTip purification protocol does not add salt), so the amount of salt present in the PCR buffer used would remain in the purified sample. Capillary electrophoresis can be salt sensitive but if your system can tolerate the amount of salt in your PCR buffer, the RapidTip may be able to save you some purification time! We are planning future products with the ability to remove salt.

17. Q: Is there a limit on DNA concentration for RapidTip effectiveness?

A: We have tested dsDNA from about 25 ng/uL to 500 ng/uL and do not see reduced performance at either end of this range. While some customers have satisfactory purification of 10 ng/uL samples, we recommend that customers purify PCR amplified DNA that is at least 25 ng/uL for best results.

18. Q: What is the retention mechanism for the particles in the tips? Is there measureable leakage or leaching of particles from the tips?

A: Diffinity technology is retained in the RapidTip with two retainers that effectively act as filters. The retainers have been optimized to retain Diffinity particles inside the tip and extensive testing has shown no measurable leakage of particles during normal use.

19. Q: Can the Diffinity RapidTip be stored in the laminar flow for UV sterilization (Does the UV light hurt the particles inside?

A: We have not tested RapidTip stability in UV light. UV light degrades polypropylene plastic over time. After some time, you can expect the plastic box to become brittle. We do not recommend long-term storage of the RapidTip in UV light. However, we believe that short exposure (1-3 hours) to UV light probably won't harm the RapidTip

21. Q: Does repeatedly opening and closing the RapidTip box cause contamination of the product?

A: Most of our customers store the RapidTip on the bench and open/close as needed without experiencing contamination. This is comparable storage to other post-PCR purification kits such as spin columns.

22. Q: I am observing bubbling and/of foaming when aspirating my sample. Why is this occurring?

A: Bubbling and foaming can occur when aspirating too quickly or when detergents are present in the sample. For best results, pipette slowly enough to minimize bubbling and foaming.

23. Q: I am having difficulty pipetting with the RapidTip. Why is this happening?

A: Because the RapidTip is a functional tip, there is more resistance than a normal pipette tip. However, it should not be overly difficult to pipette. If it is very hard to pipette, make sure your pipette tip isn't touching the bottom of the well. If you're using a ready-load PCR mix with density increasing agents, pipetting might be difficult so consider switching to a non-ready-load PCR mix for easier RapidTip purification.

24. Q: Can the RapidTip remove dye?

A: RapidTip can remove fluorescent nucleotides and some common visible dyes but is not designed to remove loading dye from ready-load PCR mixes.

25. Q: How should the RapidTip be used to maximize purification performance?

A: The RapidTip's short purification time is due in part to the use of special particles that are dispersed throughout the entire sample during the purification process.

First, tap the box of RapidTips 1-2 times on a hard surface so that the particles will drop to the bottom of the pipette tips.

To help ensure optimum performance, we recommend several pipetting guidelines:

- On the first aspirate step, pause for 4-5 seconds after aspirating the first half of the sample to enable the particles in the RapidTip to become completely wetted before completing sample aspiration
- On subsequent aspirate steps, pause 2-3 seconds before dispensing to enable the particles to interact with the sample

The total sample aspirate and dispense cycle time should still be 60 seconds.

26. Q: Can the RapidTip be used for gel purification?

A: No. The RapidTip is not designed to remove agarose. We suggest you optimize PCR for a single band prior to RapidTip purification.

27. Q: How do I measure DNA recovery?

A: To estimate percent recovery, analyses of the samples pre-purification and post-purification are necessary. For this process, we recommend either a PicoGreen®2 type assay or visualization on agarose gel.

PicoGreen: The PicoGreen assay uses an intercalating dye to specifically quantitate only double-stranded DNA. When taking a PicoGreen reading pre-purification, PCR primers and dNTPs will not falsely inflate the reading. This enables a more accurate quantification of recovery.

Agarose Gel: In addition to PicoGreen readings, visualization of the sample pre- and post-purification on agarose gel along with a DNA Ladder for accurate size and mass estimations (quantitation) of nucleic acid fragments is recommended, but would be more subjective. For most accurate results, run both pre- and post-purified samples on the same gel to minimize differences in electrophoresis parameters and imaging processes.

Spectrophotometric analysis using Optical Density (OD) at 260 nm is discouraged because at 260 nm, in the post-purified sample, any remaining single-stranded primers and dNTPs (expected to be in very small amounts that will not affect Sanger sequencing) and other interfering chemical substances that show fluorescence at this wavelength will contribute to the overall absorbance reading, giving an incorrect quantification of desired PCR product.