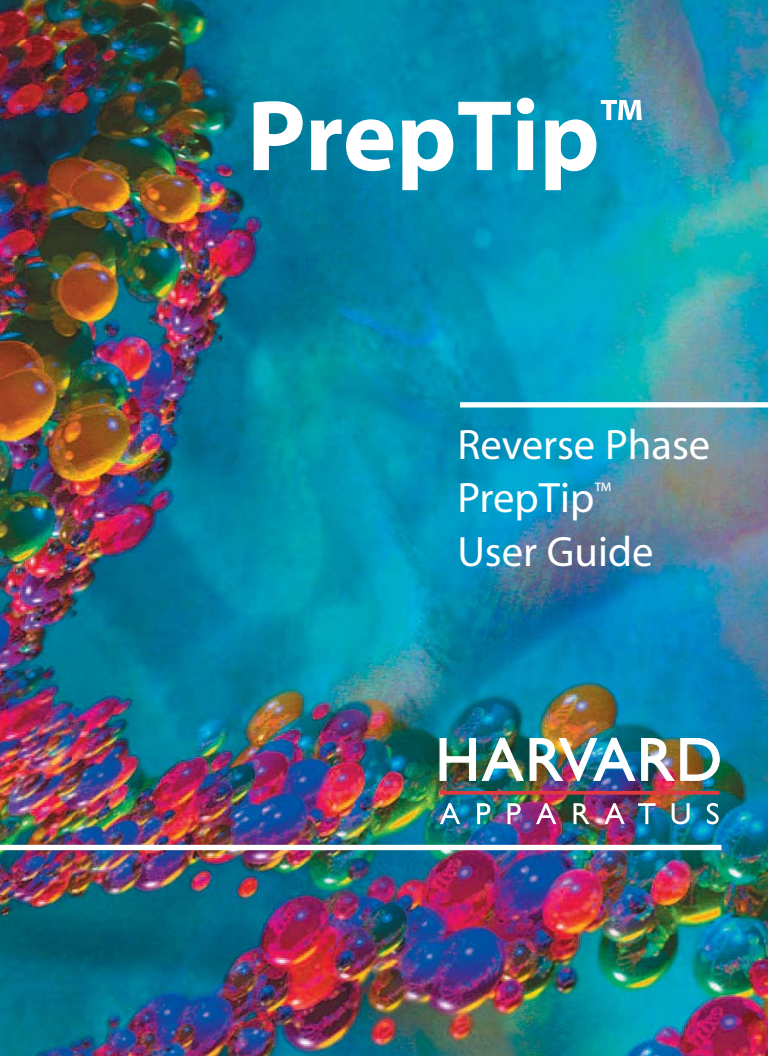
PrepTip™
Reverse Phase
PrepTip™
User Guide
HARVARD
APPARATUS

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Introduction

PrepTip™ RP from Harvard Apparatus, provides an efficient method for cleaning and concentrating proteins and other biological samples for further instrumental analysis. PrepTip's unique, patented feature is that the interior walls of the tip are coated with an inert solid matrix containing the sample-binding material. The interior coating technology makes PrepTip RP the only such product, providing both speed and reliability. Since the lower opening of the tip remains open after the column is coated the sample can flow freely through the opening without back pressure. PrepTip RP can be used to clean or concentrate any biological sample since the interior walls of the tip are coated with various binding media (pages 8–9). PrepTip RP is suited for a wide range of applications including clean-up prior to mass spectrometry and automated sample preparation methods. Sample preparation with PrepTip RP is very fast, simple and highly effective.

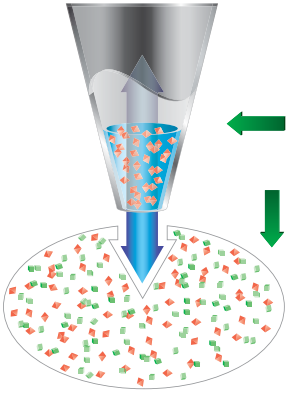
Using PrepTips™

This booklet details protocols suitable for use with our reverse phase PrepTip RP containing C4, C8, C18 or hydrophobic polymer coatings.

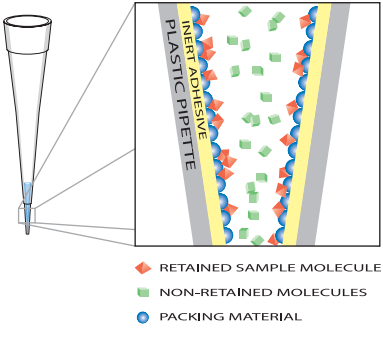
PrepTip RP are micropipette tips in which the internal surface of the tip is coated with reverse-phase binder. Proteins, peptides and other biomolecules readily bind to the surface matrix and are rapidly separated from non-hydrophobic contaminants. Biomolecules that bind to the PrepTip RP in aqueous buffers are eluted with mixtures of water and organic solvents.

Why Use PrepTip™?

The unique patented process used to coat the interior walls of PrepTip RP allows rapid diffusion of sample with minimal resistance to flow through the tip opening.

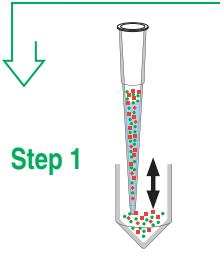


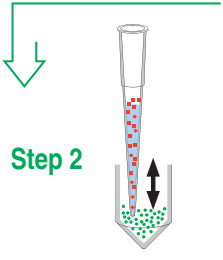
The unique process used to coat the interior walls of PrepTip RP allows sample to flow freely through the tip opening.



Begin with a mixed sample.

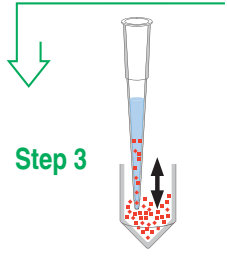
1. Aspirate the sample only once into **the tip in order to allow binding of the sample to the media**. Wait 5 seconds.

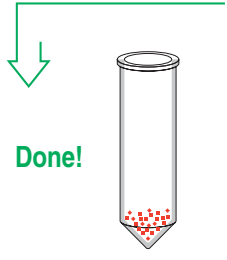




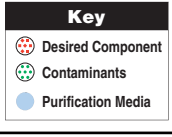
2. Wash away any unbound sample components with a suitable washing solution

3. Elute the desired sample components by aspirating a small volume of a suitable solution into and out of the tip





Purified sample in about 30 seconds!



Using the PrepTip™

Note: Like other reverse-phase media, C18 or C8 PrepTip RP may denature some large proteins during binding or elution with solvents. Consequently, for applications where biological activity must be retained, the weakly hydrophobic C4 or hydrophobic polymer PrepTip RP are recommended.

Protocol

1. Place the PrepTip RP onto a micropipette. For best results, DO NOT handle the PrepTip RP with an ungloved hand.
2. Wash the tip with a 25µl* aliquot of activating solution (100% acetonitrile). Aspirate and dispense the activating solution into and out of the tip 3 times** in order to extend the hydrophobic chains. Alternative aqueous solvents containing isopropanol or methanol are also suitable for this purpose.
3. Rinse with and equal volume of water 3 times and expel with air up to 5 times.
   Step #4 should be done immediately after step #3.
4. Using the PrepTip RP, aspirate and dispense 10µl* of sample one time** to allow the biomolecules to bind. While samples can be applied directly to the PrepTip RP, addition of 1% trifluoroacetic acid or the less hazardous phosphoric acid at a concentration of 0.1-0.3% may greatly enhance the binding of biomolecules to the RP matrix. Increasing the number of aspiration loading cycles will displace weakly binding biomolecules. Results will vary with sample composition. Its not recommended for consistent results.

* Use 25µl for 1-10µl tip & 100µl for 10-100µl tip

** 3 times for 1-10µl tip & 5 times for 10-100µl tip

5. Wash the tip twice with 10µl* of distilled water to remove any water-soluble contaminants.
6. Air dry the binder by aspirating air through the PrepTip RP with the micropipette 5-10 times.
7. Elute your sample by aspirating and dispensing a minimum volume*** of organic solvent mixture (such as 50% acetonitrile in water, 0.1% TFA or formic acid) 10 -15 times**. The concentration of organic solvent is very important for both the recovery and purity of the sample. The optimum buffer concentration must be empirically determined for each biomolecule. Tightly bound molecules will require an elution solvent of higher organic content for efficient desorption. Sample recovery can be improved by using an increased concentration of organic or by performing a second elution (although the recovered sample will be more dilute).
8. Dispose of PrepTip RP appropriately after use. Reuse is not recommended since the binding capacity is reduced and cross contamination of samples may occur.

* Use 25µl for 1-10µl tip & 100µl for 10-100µl tip

** 3 times for 1-10µl tip & 5 times for 10-100µl tip

*** Use up to 10µl for 1-10µl tip and 25µl for 10-100µl tip

Fractionation of Complex Samples

PrepTip RP columns can also be used to fractionate complex protein or peptide samples according to the following basic protocol.

Protocol

1. Place the PrepTip RP onto a micropipette. For best results, DO NOT handle the PrepTip RP with an ungloved hand.
2. Wash the tip with a 25µl* aliquot of activating solution (100% methanol). Aspirate and dispense the activating solution into and out of the tip 3 times** in order to extend the hydrophobic chains. Alternative aqueous solvents containing isopropanol or acetonitrile are also suitable for this purpose.
3. Rinse with an equal volume of water 3 times and expel with air up to 5 times.
   Step #4 should be done immediately after step #3.
4. Using the PrepTip RP, aspirate and dispense 10µl* of the sample ONE time** to allow the biomolecules to bind. While samples can be applied directly to the PrepTip RP, the addition of 1% trifluoroacetic acid or the less hazardous phosphoric acid at a concentration of 0.1% will greatly enhance the binding of biomolecules to the hydrophobic matrix. Increasing the number of loading aspiration cycles will displace weakly binding molecules. Results will vary with sample composition. Its not recommended for consistent results.

* Use 25µl for 1-10µl tip & 100µl for 10-100µl tip

** 3 times for 1-10µl tip & 5 times for 10-100µl tip

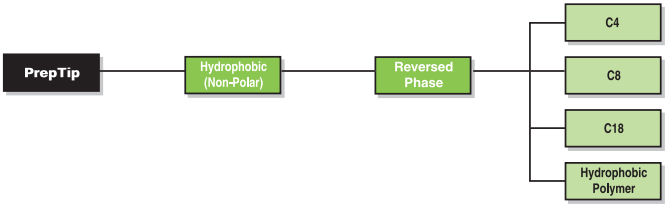
5. Wash the tip twice with 10µl* of distilled water to remove any water-soluble contaminants.
6. Dry the binder by aspirating air through the PrepTip RP with the micropipette 5-10 times.
7. Stepwise elute your sample by aspirating and dispensing a minimum volume*** of a low organic solvent contents (such as 5% acetonitrile in water, 0.1% TFA or formic acid) 10-15 times**.
8. Repeat step 6 with an increased organic content in the elution solvent (e.g. 10% acetonitrile, 0.1% TFA or formic acid). Repeat until the step gradient is completed (e.g. 5%, 10%, 20%, 30%, 40%, 50% Acetonitrile and 0.1% TFA or formic acid).
9. Dispose of PrepTip RP appropriately after use.

* Use 25µl for 1-10µl tip & 100µl for 10-100µl tip

** 3 times for 1-10µl tip & 5 times for 10-100µl tip

*** Use up to 10µl for 1-10µl tip and 25µl for 10-100µl tip

Selection Guide



Mechanism of Binding	Reverse Phase Extraction
Type of Functional Groups Bound	Nonpolar groups (e.g. aromatic and alkyl groups)
Sample Solvents Bound from...	Polar solutions (e.g. aqueous buffers)
Elute Bound Compounds with...	Polar solvents (e.g. mixtures of water with acetonitrile, or methanol)
Types of Tip Coatings Available	C4, C8, C18, Hydrophobic polymer

	Catalog Number			
Coating Materials	**C4**	**C8**	**C18**	**Hydrophobic Polymer**
1 to 10µl Tips Qty. of 24	**74-3406**	**74-3404**	SSP-UVC18F	**743418**
1 to 10µl Tips Qty. of 96	**74-3407**	**74-3405**	SSP-UVC18H	**74-3419**
10 to 100µl Tips Qty. of 24	**74-3506**	**74-3504**	**74-3502**	**74-3518**
10 to 100µl Tips Qty. of 96	**74-3507**	**74-3505**	**74-3503**	**74-3519**

Troubleshooting

Since different biomolecules will interact with different PrepTip RP in very different ways it is difficult to construct a universal protocol that will work for every sample. The basic protocol outlined in this users guide is designed to work well with the majority of sample types but will not work equally well in all cases. In some cases it will be necessary to modify this protocol to suit your desired sample prep application. The guidelines below are intended to assist you in modifying the protocol to suit your needs.

If the sample binds to PrepTip™ but is not efficiently eluted...

Potential Cause	Potential Solution
The biomolecules are not readily soluble in organic solutions.	Decrease the organic solvent concentration of the elution solvent.
Sample is very hydrophobic and is bound tightly to the surface.	Increase the organic solvent content of the elution buffer.
Protein bound too tightly to a C8, C4 or Hydrophobic Polymer PrepTip RP.	Choose PrepTip RP binder more suitable for sample. PrepTip can also be produced with custom binder coatings; contact Harvard Apparatus for details.

If the sample does not bind efficiently to PrepTip™...

Potential Cause	Potential Solution
Hydrophobic side chains not sufficiently solubilized for binding due to insufficient wetting.	Allow the wetting solution to remain in the PrepTip RP until immediately before the sample binding step.
Sample pH is too high.	Increase the concentration of TFA in the sample solution to bring the pH to below 4.0. The concentration of TFA in the sample should be between 0.1 and 1.0%.
Chemical properties of the sample do not support hydrophobic interaction with reverse-phase binder.	Choose PrepTip with binder suitable for your sample. PrepTip can also be produced with custom binder coatings, contact Harvard Apparatus for details.
Biomolecules not completely solubilized in the sample buffer.	Add Guanidine HCl to the sample buffer (final concentration 1-4M) in order to reduce secondary structure and increase access to the hydrophobic side chains on the binder surface.