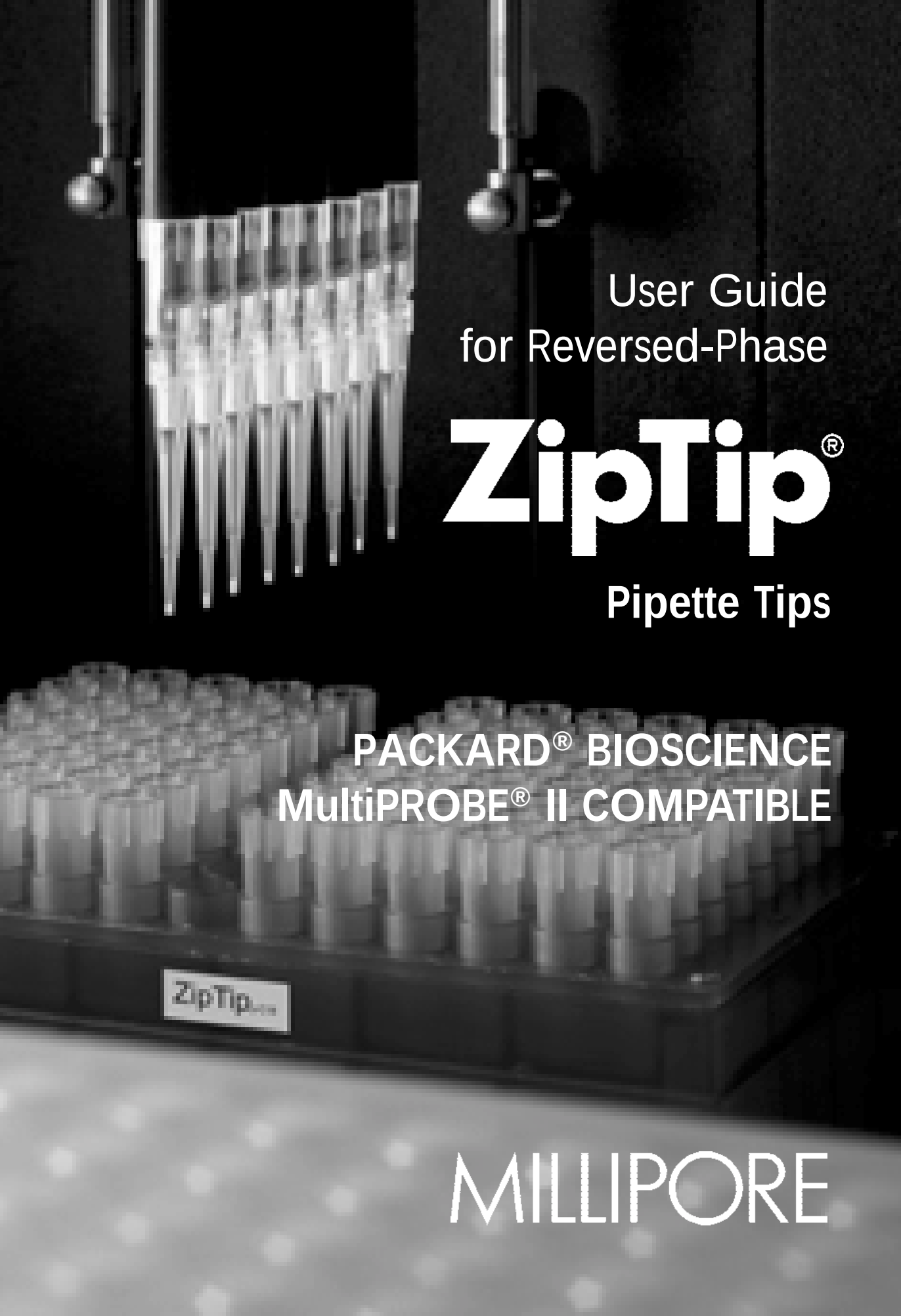

User Guide for Reversed-Phase ZipTip®

Pipette Tips

PACKARD® BIOSCIENCE MultiPROBE® II COMPATIBLE

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Notice

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Introduction

The ZipTip® pipette tip is a 10 µL pipette tip with a bed of chromatography media fixed at its end. It is ideal for concentrating and puriying peptides, proteins or oligonucleotides prior to mass spectrometry,HPLC, capillary electrophoresis, and other analytical techniques.

Millipore, in partnership with Packard BioSciences, has developed a ZipTip configuration specific for the MultiPROBE II Liquid Handling Systems. The pipette tip contains a micro bed of C18 reversed-phase resin for peptide purification and concentration.

NOTE: For specific automation protocols and technical notes, visit the Millipore automation web site: **www.millipore.com/automation**.

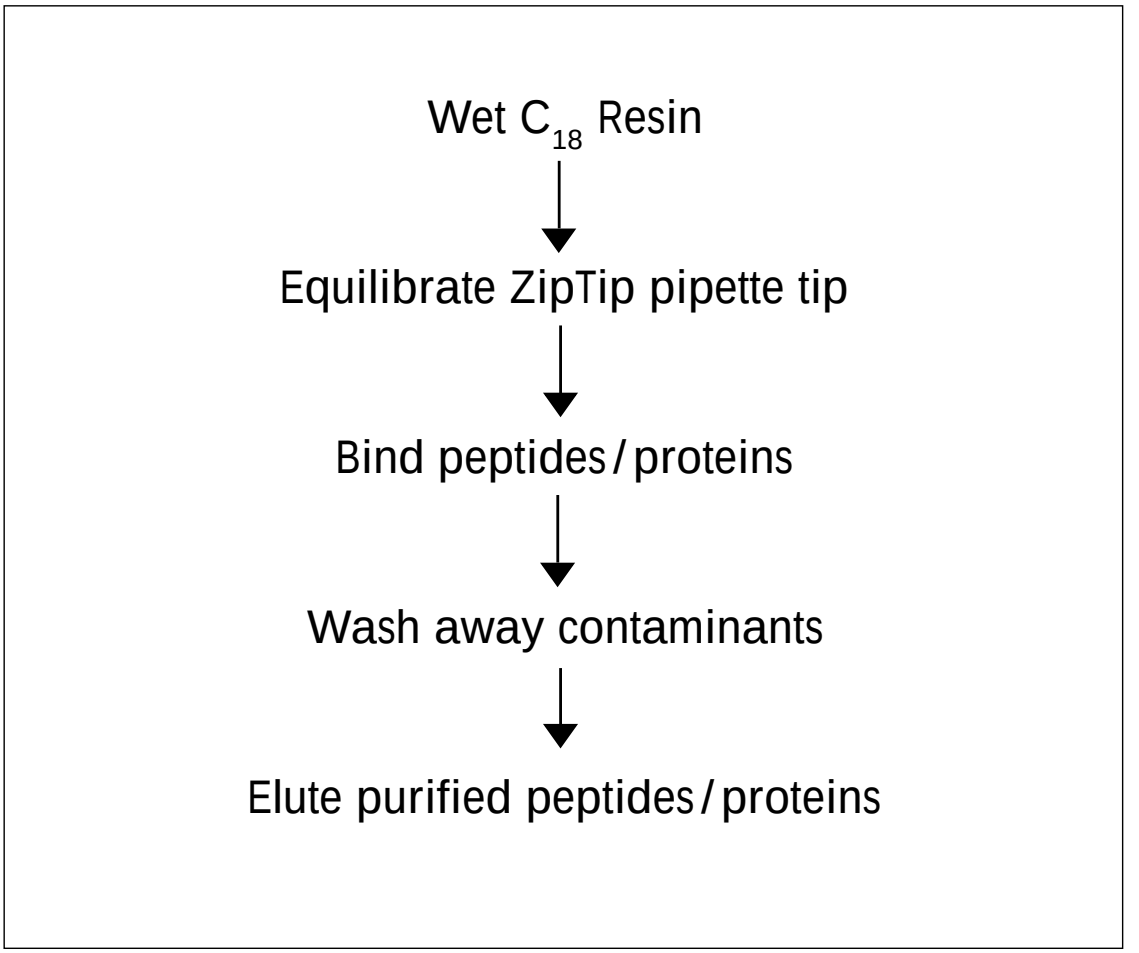
Materials

The following table outlines the solutions required for use with $ZipTip_{\mu\text{-}C18}$ pipette tips.

Solution	Description
Wetting solution	50% acetonitrile (ACN) in Milli-Q® grade water
Sample preparation solution	0.1% trifluoroacetic acid (TFA) in Milli-Q® grade water; final sample solution pH<4
Equilibration solution	0.1% TFA in Milli-Q® grade water
Wash solution	0.1% TFA in Milli-Q® grade water
Elution solution*	0.1% TFA/50% ACN, with or without MALDI matrix

** For electrospray, elute with 50% methanol in either 0.1% acetic acid or 0.1% formic acid/water.*

Overview of ZipTip Protocol Steps



Packard Deck Layout and Operation Protocols

Visit the Millipore automation web site at www.millipore.com /automation for a detailed description of the Packard BioScience MultiPROBE II system deck layout along with instrument software protocols that can be downloaded.

Chemical Compatibility

✔ = Acceptable. Long exposures at room temperature have no significant effect.

? = Questionable. Short exposures at room temperature cause little or no damage.

X = Not recommended. Short exposure may cause permanent damage.

Reagent	ZipTip
Acetic Acid (Glacial)	✔
Acetone	X
Acetonitrile (100%)	✔
Aliphatic Esters	?
Ammonium Hydroxide (5%)	✔
Benzene (100%)	X
n-Butanol (100%)	✔
Butyl Acetate	X
Chloroform (1%)	?
Dichlorobenzene (100%)	X
Dichloromethane (1%)	?
Diethanolamine (5%)	✔
Dimethyl Acetamide (100%)	X
Dimethyl Formamide (100%)	X
Dimethyl Formamide (1%)	✔
Ethanol (100%)	✔
Formic Acid (5%)	✔
Guanidine HCl (6 M)	✔
Hydrochloric Acid	✔
Hydrogen Peroxide	✔
Isopropyl Alcohol (100%)	✔
Mercaptoethanol (0.1 M)	✔
Mercaptoethanol (1.0 M)	?
Methyl Alcohol (100%)	✔
Methyl Ethyl Ketone (100%)	X
Methyl Isobutyl Ketone (100%)	✔

Chemical Compatibility, continued

Reagent	ZipTip
Nitric Acid (0.1 N)	✔
Nitric Acid (1.5 N)	✔
Phenol (0.5%)	X
Phosphoric Acid (1M)	✔
Sodium Azide (1%)	✔
Sodium Hydroxide (0.5 N)	✔
Sodium Hydroxide (0.1 N)	✔
Sodium Hypochlorite (100 ppm)	✔
Sodium Hypochlorite (200 ppm)	✔
Sulfuric Acid (1%)	✔
Toluene (1%)	X
Triton® X-100	✔
Tween®	✔
Urea (6 M)	✔

Troubleshooting

Sample preparation problems with ZipTip pipette tips can be divided into two categories:

- sample does not bind
- sample binds but is not recovered

Incomplete binding is most common. The following table outlines the most common problems and the possible causes, and suggests procedures to solve those problems. The procedures may or may not work with your ZipTip application.

For additional troubleshooting information and answers to Frequently Asked Questions, visit the Millipore Automation web site at www.millipore.com/automation.

Incomplete Binding

Possible Cause	Suggested Procedure
C_{18} beads dewetted before sample was applied. The hydrophobic beads can de-wet in less than a minute.	After wetting with ACN, flush the tip with 0.1% TFA and leave the plug immersed in liquid until immediately before sample binding.
Sample was not sufficiently acidified with TFA. The pH should be below 4. The TFA concentration should be between 0.1–1.0%.	Spike sample with a few microliters of 0.5–1% TFA.

Incomplete Elution

Possible Cause	Suggested Procedure
Sample tenaciously adsorbed to the C_{18} particles.	Increase acetonitrile content of desorption solution to a maximum of 75–90% ACN (v/v) in 0.1% TFA.
Sample not freely soluble in ACN.	Decrease ACN concentration to 20–40% in a 0.1% TFA or suitable ion-pairing agent.

Specifications

Materials of Construction

Pipette tip: Polypropylene

Media: C_{18}: spherical silica, 15 µm, 200Å pore size

Tip volume capacity 25 µL

Adsorptive bed 0.2 µL

Length 46 mm (1.825 in.)

Capacity (when used with saturating amounts of analyte): typically 2.0 µg

Max. Temperature 70 °C

Min. Temperature 4 °C

Working pH Range 2 to 13

Ordering Information

ZipTip Pack	Resin Type	Catalogue Number
96 pack, tip rack	C_{18}(micro bed)	ZTC1 8P0 96

For a complete listing of available ZipTip chemistries, visit www.millipore.com/ziptip or contact your local Millipore office.

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., look in the Millipore laboratory catalogue for the phone number of the Millipore office nearest you. You can also e-mail us at tech_service@millipore.com or visit our website (www.millipore.com).

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