

**Thermo Scientific
Aspire RP30
Desalting Pipette Tips**

User Manual



Thermo Scientific Aspire RP30 Desalting Tips

Catalog number
990-01

Description
Thermo Scientific Aspire RP30 Desalting Tips
(96 purifications)

Introduction

The Thermo Scientific Aspire RP30 Desalting Tips purify, desalt and concentrate peptides or low molecular weight proteins (65 kDa or less). The Aspire Tip is a 200 μ l chromatography pipette tip which contains a bed of proprietary dehydrated porous Styrene-divinylbenzene (SDVB) reversed-phase resin. This SDVB resin offers superior overall peptide binding and recovery compared to traditional C18 products.

Mass spectrometry methods are becoming important tools for the study of biological compounds. Salts, detergents and other contaminants present in biological samples have deleterious effects on the quality of MS spectra. Chromatography tips are convenient for peptide desalting and have been widely used for Matrix-assisted laser desorption/ionization (MALDI) sample cleanup and concentration. As researchers begin to analyze samples of increasing complexity, sample cleanup using conventional desalting tips becomes less ideal due to their low binding capacity (1-5 μ g) and recovery. The Aspire RP30 Desalting Tips allow purification of biological samples, including complex mixtures containing up to 30 μ g of total peptides, rendering them ideal for pre- LC/MS sample cleanup. The Aspire Tips effectively remove salts, detergents and other ion-suppressing interference from biological samples prior to LC/MS and LC/MS/MS analysis, improving sensitivity and data quality.

The color-coded, multichannel purification protocol is optimized for low to medium-throughput sample cleanup. The Aspire tips are compatible with a variety of handheld pipettes. For the user's convenience, the procedure is further optimized on a Thermo Scientific Finnpiquette[®] Novus electronic multichannel pipette (see page 4 for instructions). Twelve samples can be processed in parallel within 20 minutes without the need for centrifugation.

Kit Contents

- **Aspire RP30 Desalting Tips (96 tips)**
- **Assorted color-coded tubes**
- **Tube rack (1)**
- **Tube caps (96)**
- **User manual**

Storage

Upon receipt, store all components at room temperature. Product is shipped at ambient temperature.

This product is supplied for life science research use only. This product is not intended for medicinal, diagnostic or therapeutic use.

Procedure Using a Handheld Pipette

A. Additional Materials Required

- 200-300 μ l single or multichannel pipette (manual or electronic)
- Ultrapure water
- Acetonitrile (ACN)
- Trifluoroacetic acid (TFA)
- Methanol

B. Material Preparation

- Activation Solution: 50% Methanol. Note: ACN can be substituted for methanol.
- Equilibration/Wash Solution: 0.5% TFA in 5% ACN
- Sample Solution: 2% TFA in 20% ACN
- Elution Solution: 70% ACN

C. Purification Protocol

Note: Perform the following procedure at room temperature.

1. Arrange the color-coded tubes in the provided rack as illustrated in Diagram 1. Each sample requires the following quantities of color-coded tubes for processing:
 - 1 blue
 - 3 green
 - 1 yellow
 - 2 orange

Note: Up to 12 samples can be processed in parallel on a multichannel pipette.

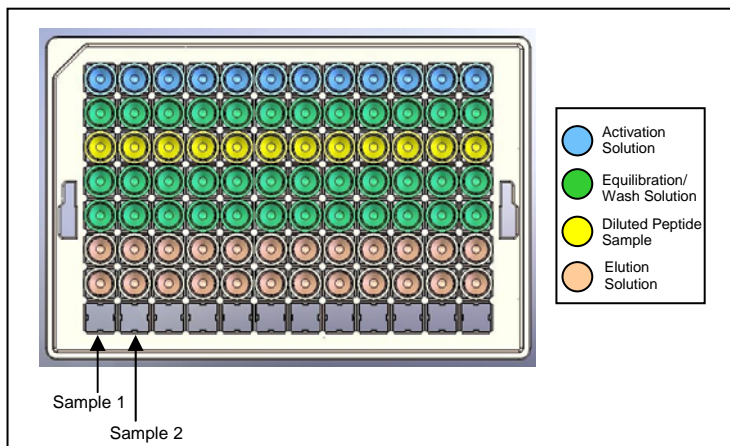


Diagram 1: Tube arrangement for processing of twelve samples

2. Aliquot 100 μ l of Activation Solution into each blue tube.
3. Aliquot 100 μ l of Equilibration/Wash Solution into each green tube.
4. In each yellow tube, mix 3 parts sample to 1 part Sample Solution. The final sample will contain 0.5% TFA in 5% ACN. *For example:* Add 25 μ l of Sample Solution to 75 μ l of sample.
5. Aliquot a minimum of 25 μ l of Elution Solution into each orange tube. The recommended range of elution volumes is 25-50 μ l.
6. Adjust pipette setting to 100 μ l and attach Aspire Tip(s) to the pipette.
7. Completely re-hydrate and activate resin inside the Aspire tip(s) by aspirating 100 μ l of Activation Solution from the blue tube (row A). Expel solution back into the same tube. Repeat once with the same fraction of Activation Solution (row A).
8. Insert the Aspire tip(s) into the green tube (row B). Equilibrate resin by aspirating 100 μ l of Equilibration/Wash Solution and expel solution back into the same tube. Repeat once with same fraction of Equilibration/Wash Solution (row B).
9. Adjust pipette setting to sample volume and insert Aspire tip(s) into the yellow tube (row C). Bind peptides by aspirating and dispensing diluted sample at least 6 times. Expel flow-through back into the same tube.
Note: Retain flow-through fraction if verification of sample binding is desired.
10. Adjust pipette setting to 100 μ l. Insert Aspire tip(s) into the second green tube (row D). Wash resin by aspirating 100 μ l of Equilibration/Wash solution. Expel solution to same tube. Repeat once with same fraction of Equilibration/Wash Solution (row D).
11. Insert Aspire tip(s) into the third green tube (row E). Repeat wash step outlined in step 10.
12. Adjust pipette setting to 5 μ l greater than the elution fraction volume. *For example:* Adjust pipette setting to 30 μ l for the elution fraction volume of 25 μ l. Insert Aspire tip(s) into the orange tube (row F). Elute peptides by aspirating and dispensing Elution Solution 5 times. Dispense eluate back into the same tube.
Note: Although bubbling may occur during these cycles, it will not affect sample recovery.
13. Continue elution by inserting Aspire tip(s) into the orange tube (row G). Aspirate and dispense Elution Solution 5 times. Dispense eluate back into the first orange tube in row F to pool the two elution fractions. The orange tubes can be securely sealed with the provided caps.
14. If needed, gently dry sample in a vacuum evaporator to further concentrate eluate and lower the acetonitrile concentration for downstream applications. To dry eluate, carefully insert the orange tube (pooled elution fraction) without the cap into a 1.5 or 2.0 ml centrifuge tube and proceed drying in a vacuum evaporator.
15. For LC/MS applications, resuspend sample in 0.1% formic acid or the appropriate solution after drying. For MALDI-MS analysis, carefully resuspend sample in 1-2 μ l of matrix solution prepared just before use.

Procedure Using a Thermo Scientific Finnpipette[®] Novus Electronic Multichannel

A. Additional Materials Required

- 200-300 µl single or multichannel electronic Novus pipette
- Ultrapure water
- Acetonitrile (ACN)
- Trifluoroacetic acid (TFA)
- Methanol

B. Material Preparation

- Activation Solution: 50% Methanol. Note: ACN can be substituted for methanol.
- Equilibration/Wash Solution: 0.5% TFA in 5% ACN
- Sample Solution: 2% TFA in 20% ACN
- Elution Solution: 70% ACN

C. Purification Protocol

Note: Perform the following procedure at room temperature.

1. Arrange the color-coded tubes in the provided tube rack as illustrated in Diagram 1 (page 2). Each sample requires the following quantities of color-coded tubes for processing:
 - 1 blue
 - 3 green
 - 1 yellow
 - 2 orange

Note: Up to 12 samples can be processed in parallel on a multichannel pipette.

2. With the Finnpipette Novus electronic multichannel pipette, aliquot 100 µl of Activation Solution into each blue tube.
3. Aliquot 100 µl of Equilibration/Wash Solution into each green tube.
4. In each yellow tube, mix 3 parts sample to 1 part Sample Solution. The final sample will contain 0.5% TFA in 5% ACN. *For example:* Add 25 µl of Sample Solution to 75 µl of sample.
5. Aliquot a minimum of 25 µl of Elution Solution into each orange tube. The recommended range of elution volumes (per tube) is from 25-50 µl.
6. Using the **STEPPER** function on the Novus pipette, adjust pipette setting to 100 µl. Adjust the aspirate and dispense rates to 9 on the Novus pipette. Attach Aspire RP30 Desalting Tip(s) to the pipette.

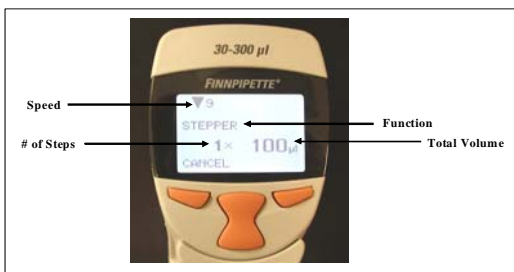
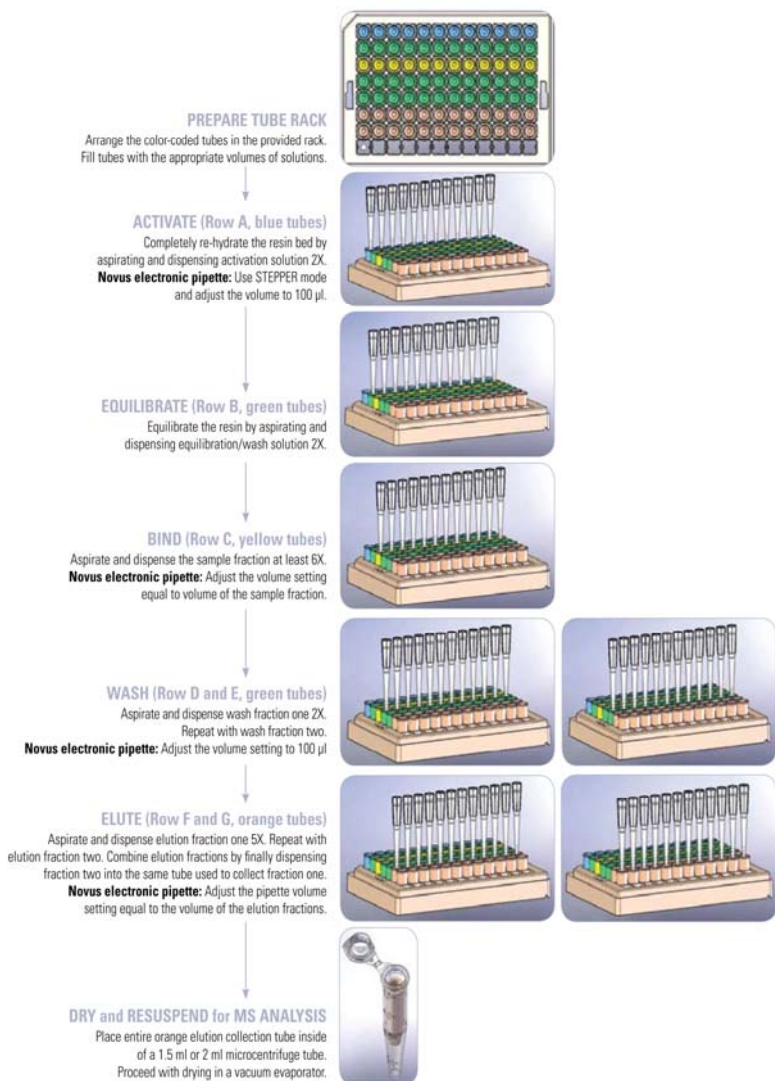


Figure 1: Finn pipette Novus electronic multichannel *STEPPER* function settings

Note: The *STEPPER* function is used as it allows for a *blowout* step which ensures complete dispensing of all aspirated liquid.

7. Completely re-hydrate and activate resin inside the Aspire tip by aspirating 100 µl of Activation Solution from the blue tube (row A). Expel solution back to the same tube. Repeat once with same fraction of Activation Solution (row A).
Note: Hold the trigger button down during the *blowout* step until all of the liquid is expelled from the tip. The *dispensing* step only expels a small portion of the liquid, the *blowout* step subsequently expels the remainder of the liquid from the tip.
8. Insert Aspire tip(s) into the green tube (row B). Equilibrate resin by aspirating 100 µl of Equilibration/Wash Solution and expel solution back into the same tube. Repeat once with same fraction of Equilibration/Wash Solution (row B).
9. Keeping the speed and *STEPPER* mode, adjust pipette volume setting equal to the sample volume and insert Aspire tip(s) into the yellow tube (row C). Bind peptides by aspirating and dispensing diluted sample at least 6 times. Expel flow-through back into the same tube.
Note: Retain flow-through fraction if verification of sample binding is desired.
10. Keeping the speed and *STEPPER* mode, change pipette to the volume setting to 100 µl. Insert Aspire tip(s) into the second green tube (row D). Wash resin by aspirating 100 µl of Equilibration/Wash solution. Expel solution to same tube. Repeat once with the same fraction of Equilibration/Wash Solution (row D).
11. Insert Aspire tip(s) into the third green tube (row E). Repeat wash step outlined in step 10.
12. Keeping the speed and *STEPPER* mode, adjust pipette volume setting equal to the elution volume.
13. Insert Aspire tip(s) into the orange tube (row F). Elute peptides by aspirating and dispensing Elution Solution 5 times. Dispense eluate back into the same tube.
Note: Although bubbling may occur during these cycles, it will not affect sample recovery.
14. Continue elution by inserting Aspire tip(s) into the orange tube (row G). Aspirate and dispense Elution Solution 5 times. Dispense eluate into the first elution fraction in order to pool the two fractions together. The orange tubes (row F) can be securely sealed with the provided caps.
15. If needed, gently dry sample in a vacuum evaporator to further concentrate eluate and lower the acetonitrile concentration for downstream applications. To dry eluate, carefully insert the orange tube (pooled elution fraction) without the cap into a 1.5 or 2.0 ml centrifuge tube and proceed drying in a vacuum evaporator.
16. For LC/MS applications, resuspend sample in 0.1% formic acid or the appropriate solution after drying. For MALDI-MS analysis, carefully resuspend sample in 1-2 µl of matrix solution prepared just prior to use.

D. Procedure Flowchart



Troubleshooting

Problem	Potential Cause	Solution
Poor or incomplete sample binding	High pH, lack of ion-pairing agents	Ensure sample is diluted in Sample Solution with TFA
	Sample contains organic solvent	Dry sample and resuspend in 20 µl 0.1-0.5% TFA
	Peptides are too hydrophilic to bind resin	Increase TFA concentration from 0.5% to 1% in the sample binding step
Poor or incomplete sample recovery	Highly hydrophobic sample	Use 70%-95% ACN/ 0.1% TFA for elution conditions
	Sample contains hydrophilic peptides or a wide range of peptides	Increase TFA concentration from 0.5% to 1% in the sample binding step

Related Thermo Scientific Products

4630040X* **Thermo Scientific Finnpipette® Novus Multichannel Electronic Pipette** (8-channel, 30-300 µl)

4630050X* **Thermo Scientific Finnpipette® Novus Multichannel Electronic Pipette** (12-channel, 30-300 µl)

* X: 0=Eur, 1=U.S., 2=Japan, 3=U.K. and 4=Australia

MSDS Information

The Material Safety Data Sheet (MSDS) information for the products is provided on www.thermo.com. Simply navigate to the product page to retrieve any associated MSDS's in a print format. MSDS documents are not included with product shipments.

Expiration Period

One year from date of sale for product used, handled and stored according to Manufacturer instructions, see details under **Warranty**.

The most current versions of all Aspire product instructions are available at www.thermo.com. For a copy, please go to www.thermo.com or your local distributor.

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