# Thermo Scientific Aspire RP30 Desalting Tips

| Catalog number | Description |
|---|---|
| 990-01 | **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 µl 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 µg) and recovery. The Aspire RP30 Desalting Tips allow purification of biological samples, including complex mixtures containing up to 30 µg 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 Finnpipette® 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.

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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 µl 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.

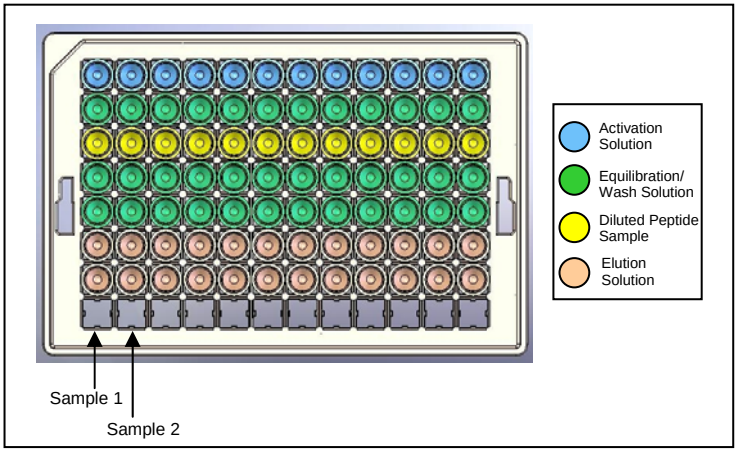



**Diagram 1:** Tube arrangement for processing of twelve samples

2. Aliquot 100 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 is 25-50 µl.

6. Adjust pipette setting to 100 µl and attach Aspire Tip(s) to the pipette.

7. Completely re-hydrate and activate resin inside the Aspire tip(s) by aspirating 100 µl 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 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. 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 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 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 µl greater than the elution fraction volume. *For example*: Adjust pipette setting to 30 µl for the elution fraction volume of 25 µl. 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 µl 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)
- Trifluoroactic 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.

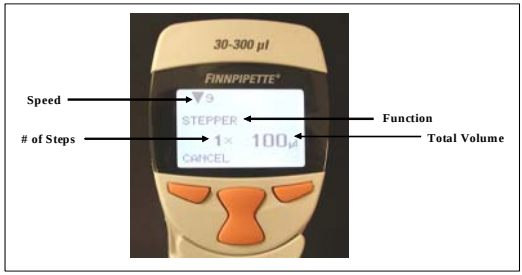



**Figure 1:** Finnpipette Novus electronic multichannel ***STEPPER*** function settings

**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.

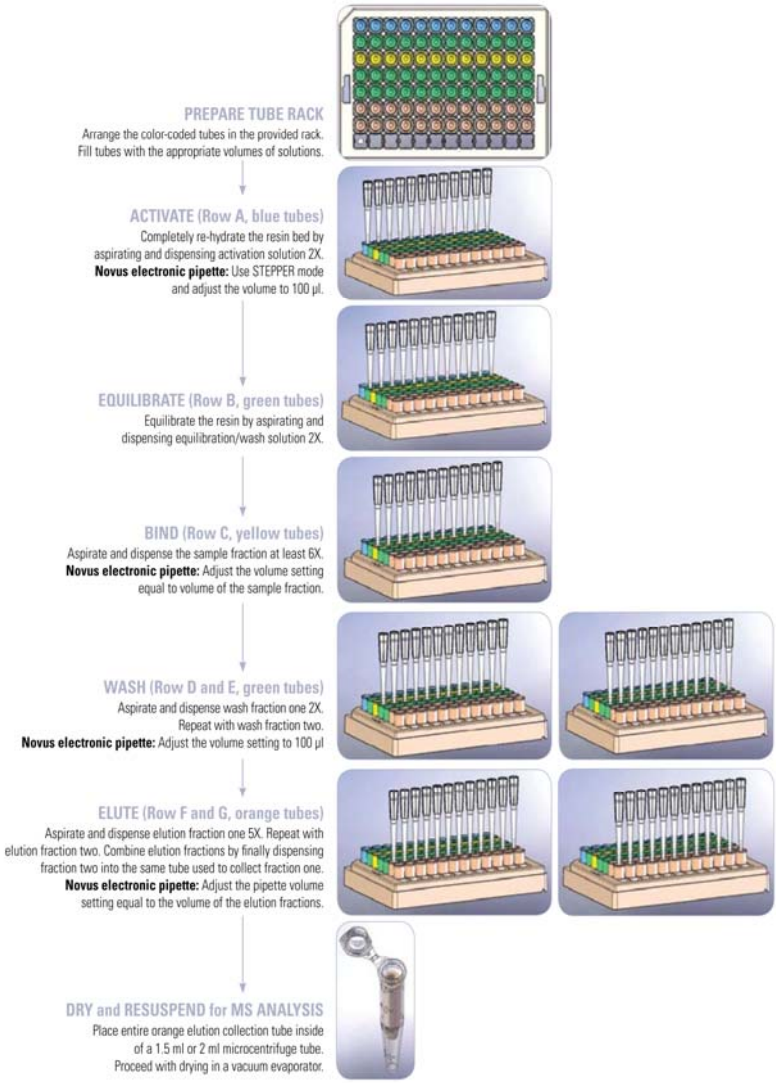
13. Insert Aspire tip(s) into the orange tube (row F). Elute peptides by aspirating and dispensing Elution Solution 5 times. Dispense eluate back into the same tube.
   **Note:** Although bubbling may occur during these cycles, it will not affect sample recovery.

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.

**Warranty:** Sellers warrants that the Products will operate or perform substantially in conformance with Seller's published specifications and be free from defects in material and workmanship, when subjected to normal, proper and intended usage by properly trained personnel, for the period of time set forth in the product documentation, published specifications or page insert. If a period of time is not specified in Seller's product documentation, published specifications or package inserts, the warranty period shall be one (1) year from the date of shipment to Buyer for equipment and ninety (90) days for all other products (the "Warranty Period"). Seller agrees the Warranty Period, to repair or replace at Seller's option, defective Products so as to cause the same to operate in substantial conformance with said published specifications, provided that Buyer shall (a) promptly notify Seller in writing upon the discovery of any defect, which notice shall include the product model and serial number (if applicable) and details of the warranty claim, and (b) after Seller's review. Seller will provide Buyer with service data and/or a Return Material Authorization ("RMA"), which may include biohazard decontamination procedures and other product-specific handling instructions, then, if applicable, Buyer may return the effective Products to Seller with all costs prepaid by Buyer. Replacement parts may be new or refurbished at the election of Seller. All replaced parts shall become the property of Seller. Shipment to Buyer repaired or replacement Products shall be made in accordance with the Delivery provisions of the Seller's Term and Conditions of Sale. Consumables are expressly excluded this warranty.

Notwithstanding the foregoing, Products supplied by Seller that are obtained by Seller from an original manufacturer or third party supplier are not warranted by Seller, but Seller agrees to assign to Buyer any warranty rights in such Product that Seller may have from the original manufacturer or third party supplier, to the extent such assignment is allowed by such original manufacturer or third party supplier.

In no event shall Seller have any obligation to make repairs, replacements or corrections required, in whole or in part, as the result as (i)normal wear and tear, (ii)accident, disaster or event of force majeure, (iii)misuse, fault or negligence of or by Buyer, (iv)use of the Products in a manner for which they were not designed, (v)causes external to the Products such as, but not limited to, power failure or electrical power surges, (vi)improper storage and handling of the Products or (vii) use of the Products in combination with equipment or software not supplied by Seller. If Seller determines that Products for which Buyer has requested warranty services are not covered by the warranty hereunder. Buyer shall pay or reimburse Seller for all costs of investigating and responding to such request at Seller's then prevailing time and materials rates. If Seller provides repair services or replacement parts that are not covered by this warranty, Buyer shall pay Seller therefore at Seller's then prevailing time and materials rates. Any installation, maintenance, repair, service, relocations or alteration to or of, or other tampering with, the products preformed by any person or entity other than seller without sellers's prior written approval, or any use of replacement parts not supplied by seller, shall immediately void and cancel all warranties with respect to the affected products. The obligations created by this warranty statement to repair or replace a defective product shall be the sole remedy of buyer in the event of defective product except as expressly provided in this warranty statement, seller disclaims all other warranties, whether express or implied, oral or written, with respect to the products, including without limitations all implied warranties of merchantability or fitness for any particular purpose. Seller does not warrant that the products are error-free or will accomplish any particular result.