PROTOCOL: Reversed Phase Peptide Desalt (RPS)

Purpose

To remove salts from samples prior to mass spectrometric analysis.

Preparation

1. Prepare RPSMIX01 (0.1% TFA)
2. Prepare RPSMIX02 (50% ACN/0.1% TFA)

Materials

- HPLC-grade water, JT Baker, Cat. No. 4218-03 {RPS-M01}
- Acetonitrile, EMD Millipore, Cat. No. AX0156-1 {RPS-M02}
- Trifluoroacetic Acid, Sigma-Aldrich, Cat. No. T6508-25ML {RPS-M03}
- 500uL V-bottom plate, VWR, Cat. No. 89005-016 {RPS-M04}
- 96-Well Hard Shell, skirted PCR plate, Bio-rad, Cat. No. HSP9601 {RPS-M05}
- 96-Well Round Bottom Microplate, Greiner Bio-One, Cat. No. 650101 {RPS-M06}
- Agilent AssayMap Bravo RP-S Cartridges, Agilent Technologies, Cat. No. G5496-60033 {RPS-M07}
- Breathable Seal, Diversified Biotech, Cat. No. BERM-2000 {RPS-M08}
- Axygen -80°C Rated Foil Seal, Axygen, Cat. No. PCRAS200 {RPS-M09}
- 1-Well Low Profile Reagent Reservoir, Axygen, Cat. No. RES-SW1-LP {RPS-M10}
- 500uL Micronic Vials (Snap Tubes) in Loborack, Micronic, Cat. No. 1754-2072 {RPS-M11}

Assets

- Agilent AssayMap-BRAVO Automated Liquid Handling Platform with VWorks4 {RPS-A01}
- Thermo Scientific Savant SC210A Concentrator {RPS-A02}

Reagent Mixes

ID	Name	Step	Composition	Volume/Well	Use
RPSMIX01	0.1% TFA (Wash Buffer)	**RPS**	0.1% TFA {RPS-M03} in HPLC grade water {RPS-M01}	480uL [355]	Wash and equilibrate RP-S cartridges. CAUTION ACID
RPSMIX02	50% ACN/0.1% TFA (Elution Buffer)	**RPS**	50% acetonitrile {RPS-M02}/0.1% TFA { RPS-M03} in HPLC-grade water	480uL [350]	Elute peptides from RP-S cartridges.

Mix Preps and Mini-worksheets:

RPSMIX01 – 0.1% TFA

1. Measure 999 mL of HPLC-grade water {RPS-M01} in a graduated cylinder and

add to a 1L bottle. ❑

2. Pipette 1mL of TFA {RPS-M03} into the bottle. ❑

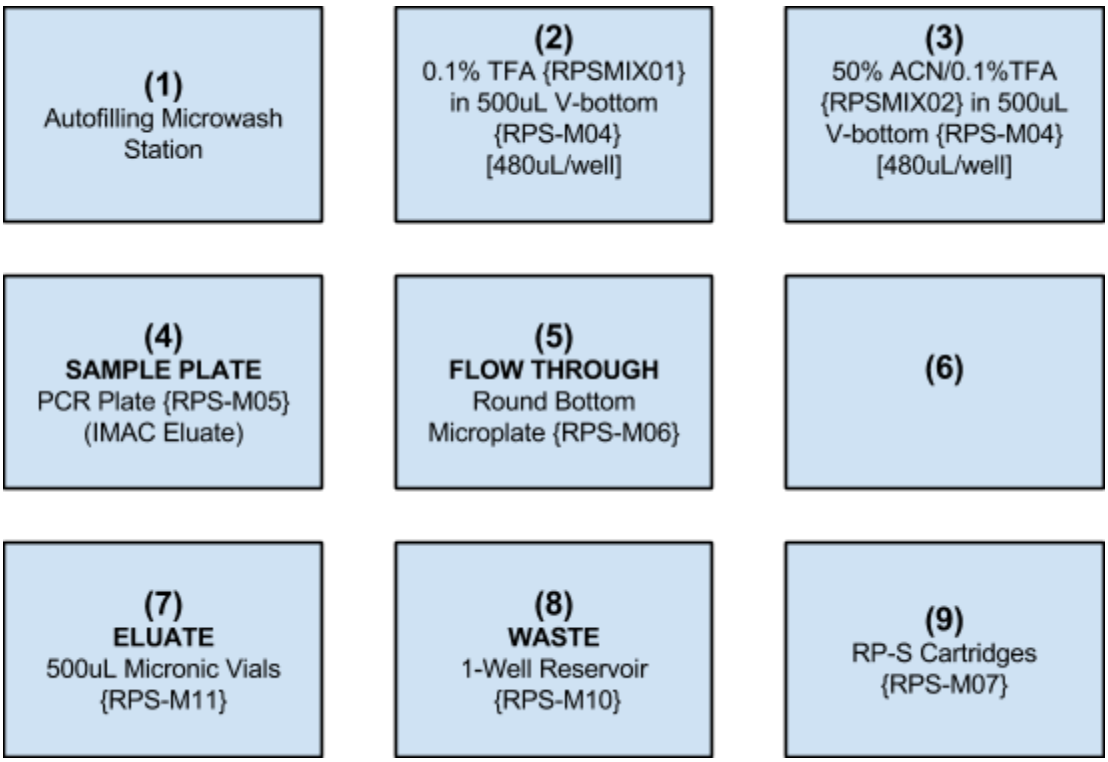
RPSMIX02 – 50% ACN/0.1% TFA

1. Measure 500mL of HPLC-grade water {RPS-M01} in a graduated cylinder and add to a 1L bottle. ❑
2. Measure 500mL of acetonitrile {RPS-M02} in a graduated cylinder and add to the bottle. ❑
3. Pipette 1mL Trifluoroacetic acid {RPS-M03} into the bottle. ❑

Procedure

1. Fill a 500uL V-bottom plate {RPS-M04} with 480uL of 0.1%TFA {RPSMIX01} [355uL required].
2. Fill a 500uL V-bottom plate { RPS-M04} with 480uL of 50% ACN/0.1% TFA {RPSMIX02} [350uL required].
3. Fill the Wash Station reservoir with 0.1% TFA {RPSMIX01} and ensure that the tubing is fully submerged. Check the waste container to see if the tubing leading into it is still above liquid level. If the container is full, dispose of the waste in the correct satellite waste container
4. Prepare AM-BRAVO for operation:
    - 4.1. On the AM-BRAVO Eva {IMAC-A01} load the device file “AssayMap Bravo_CF.dev”. This file is located at C:\\VWorks Workspace\Device Files\.
    - 4.2. In the “Devices” page, click on “Agilent Bravo” and then “AM-EVA”. Select “Initialize all devices”.
    - 4.3. Open the protocol file “3.0_RP-S_Bind Wash Elute_CF_V4.pro”. This file is located at C:\\VWorks Workspace\Protocol Files\AM-Bravo\IMAC\.
5. Assemble the deck of the LT-BRAVO according to the following layout:

Note: The **SAMPLE PLATE** in position 4 is the **IMAC ELUATE** plate from the previous protocol, **Automated IMAC Enrichment (IMAC).**



6. On the AM-BRAVO, toggle to “Simulation is on” at the top of the screen from “Simulation is off”.
    - 6.1. Press Start and the Run Configuration Wizard will pop up. Press Finish.
    - 6.2. A pop up entitled “Set Initial Values for Variables” will appear. Set the number of “CartridgeColumns” to the appropriate amount of sample columns. (RPS Automation Protocol Step 1).

6.3. Change the values for the other parameters listed if necessary and press ok.

6.4. The simulation will run and provide feedback on any warnings or errors that the protocol may encounter. If there are any **unknown** errors that come up, notify the key AM-BRAVO user and obtain help.

NB: There will always be errors and warnings when running this protocol. A list of the expected errors and warnings can be found at the end.

7. On the AM-BRAVO, toggle back to “Simulation is off”. Follow steps 6.1 to 6.3 in order to run the protocol. (RPS Automation Protocol Steps 2-8).
8. When the protocol is finished, clear the deck.
    8.1. Dispose of waste in the “Waste” reservoir at the appropriate satellite accumulation station.
    8.2. Retain any labware that can be reused. Empty appropriately, rinse with water, and leave to dry.
9. Upon completion of the protocol, note the condition of samples and seal plates to be saved.
    9.1. No volume should remain in the **SAMPLE PLATE** in position 4. Seal this plate with foil {RPS-M09} and store at -80C.
    9.2. Place foil {RPS-M09} on the **FLOW THROUGH** plate and transfer to -80C.
    9.3. Create a **Balance** plate by pipetting 50uL of 50% ACN/0.1% TFA {RPSMIX02} into each vial of a Micronic Vials Loborack {RPS-M11}.
    9.4. Cover the RP-S **ELUATE** plate in position 7 with foil {RPS-M09}, vortex, spin down, and remove the seal.
    9.5. Cover both RP-S **ELUATE** and **Balance** plates with a breathable seal {RPS-M08}, then foil {RPS-M09}, and freeze at -80°C.
    9.6. Remove the foil seals from the RP-S **ELUATE** and **Balance** plate and speedvac to dryness.
        9.6.1.1. Once dry, keep peptides at 4°C if analyzing immediately or freeze at -80°C.

Known Errors:

Main Log

Timestamp	Class	Device	Location	Process	Task	Description
11/30/2015 10:45:58 AM	Info					Process window minimized
11/30/2015 10:45:59 AM	Info					Process window un-minimized
11/30/2015 10:50:43 AM	Info					Process window minimized
11/30/2015 10:54:43 AM	Info					Compile protocol
11/30/2015 10:54:48 AM	Error	AM-EVA		Cup Washing	6	Attempting to use cartridges-off while there are no cartridges on the head.
11/30/2015 10:54:48 AM	Error	AM-EVA		Cup Washing	6	75 µL in the tips when the tips are being pushed off.
11/30/2015 10:54:48 AM	Error	AM-EVA		Cup Washing	6	Attempting to use cartridges-off while there are no cartridges on the head.
11/30/2015 10:54:48 AM	Error	AM-EVA		Cup Washing	6	75 µL in the tips when the tips are being pushed off.
11/30/2015 10:54:48 AM	Error	AM-EVA		Cup Washing		A Cartridges-On task should be paired by Cartridges-Off inside a loop.
11/30/2015 10:54:48 AM	Error	AM-EVA		Elution	5	Pipetting with cartridges on the 96AM head too deep may cause crash.
11/30/2015 10:54:48 AM	Error	AM-EVA		Sample Loading	2	Pipetting with bare probes on the 96AM head too deep may cause crash.
11/30/2015 10:54:48 AM	Error	AM-EVA		Sample Loading	7	5 µL in the tips when the tips are being pushed off.
11/30/2015 10:54:48 AM	Info					Compile complete with 8 errors and 0 warnings
11/30/2015 10:55:03 AM	Info					File loaded

RPS Automation Steps (BRAVO-AssayMAP)

1. Define Variables
    1.1. CartridgeColumns = 12
    1.2. ElutionRate = 0.083
    1.3. ElutionVolume = 100
    1.4. EquilibrationRate = 0.417
    1.5. EquilibrationVolume = 50
    1.6. NumberOfCupWashes = 3
    1.7. PrimingRate = 5
    1.8. SampleRate = 0.033
    1.9. SampleVolume = 50
    1.10. WashLoops = 3
    1.11. WashRate = 0.417
    1.12. WashVolume = 50

2. Priming
    2.1. Set head mode to all barrels
        2.1.1. task.Headmode="1,2,8,"+CartridgeColumns;
        2.1.2. This script can be used in "Advanced Settings" in conjunction with "Define Variables" to set the number of "Cartridge Columns" to the appropriate number.
    2.2. Wash tips with 240uL at Position 1. (Wash Station)
        2.2.1. Liquid class = AM_50uLperSec
        2.2.2. Mix cycles = 1
    2.3. AM Aspirate 150uL from Position 3. (Elution Buffer)
        2.3.1. Liquid class = AM_100uLperSec
        2.3.2. Distance from well bottom = 3
    2.4. AM Cartridges on at Position 9.
    2.5. AM Dispense contents of tips to Position 8. (Organic Waste)
        2.5.1. Liquid class = AM_25uLperSec
        2.5.2. Dispense flow rate = PrimingRate
        2.5.3. Distance from well bottom = 5
    2.6. AM Cartridges off at Position 9.

3. Equilibration
    3.1. Set head mode to all barrels
        3.1.1. task.Headmode="1,2,8,"+CartridgeColumns;
        3.1.2. This script can be used in "Advanced Settings" in conjunction with "Define Variables" to set the number of "Cartridge Columns" to the appropriate number.
    3.2. Wash tips by emptying contents of tips to Position 1. (Wash Station)
        3.2.1. Liquid class = 2_5sec Delay
        3.2.2. Distance from well bottom = 20
    3.3. Move above location 1.
    3.4. Dispense to waste contents of tips at Position 1. (Wash Station)
        3.4.1. Distance from well bottom = 25
    3.5. AM Aspirate 50uL from Position 2. (Utility Buffer)
        3.5.1. Volume = EquilibrationVolume

3.5.2. Liquid class = CF_200uLperMin_10secDelay
3.5.3. Distance from well bottom = 2
3.6. AM Cartridges on from Position 9.
3.7. Dispense to waste contents of tips at Position 1. (Wash Station)
3.7.1. Liquid class = !AM_10uLperMin_0.167uLperSec
3.7.2. Dispense flow rate =EquilibrationRate
3.7.3. Distance from well bottom = 22
3.8. Wash tips with 0uL at Position 1. (Wash Station)
3.8.1. Liquid class = AM_2_5sec Delay
3.8.2. Mix cycles = 0
3.8.3. Distance from well bottom = 22
3.9. AM Cartridges off at Position 9.
3.10. Wash tips with 240uL at Position 1. (Wash Station)
3.10.1. Liquid class = AM_50uLperSec
3.10.2. Mix cycles = 1
3.10.3. Distance from well bottom = 20
3.11. Move above location 1.
3.12. Dispense to waste contents of tips at Position 1. (Wash Station)
3.12.1. Distance from well bottom = 25

4. Sample Loading
4.1. Set head mode to all barrels
4.1.1. task.Headmode="1,2,8,"+CartridgeColumns;
4.1.2. This script can be used in "Advanced Settings" in conjunction with "Define Variables" to set the number of "Cartridge Columns" to the appropriate number.
4.2. AM Aspirate 50uL from Position 4. (Sample Plate)
4.2.1. Volume =SampleVolume
4.2.2. Liquid class = CF_200uLperMin_10SecDelay
4.2.3. Distance from well bottom = 0
4.3. AM Cartridges on from Position 9.
4.4. AM Dispense contents of tips to Position 5. (Flow Through)
4.4.1. Liquid class = AM_10uLperSec
4.4.2. Dispense flow rate = SampleRate
4.4.3. Distance from well bottom = 2
4.5. Wash tips with 0uL at Position 3. (Wash Station)
4.5.1. Liquid class = AM_2_5sec Delay
4.5.2. Mix cycles = 0
4.5.3. Distance from well bottom = 22
4.6. AM Aspirate 5uL from Position 2. (Utility Buffer)
4.6.1. Liquid class = AM_10uLperSec
4.6.2. Aspirate flow rate =SampleRate
4.6.3. Distance from well bottom = 2
4.7. AM Cartridges off at Position 9.
4.8. AM Dispense contents of tips to Position 5. (Flow Through)
4.8.1. Distance from well bottom = 2
4.9. Wash tips with 240uL at Position 3. (Wash Station)
4.9.1. Liquid class = AM_50uLperSec
4.9.2. Mix cycles = 1

4.9.3. Distance from well bottom = 20
4.10. Move above location 1.
4.11. Dispense to waste contents of tips at Position 1. (Wash Station)
4.11.1. Distance from well bottom = 25

5. Cup Washing
5.1. Set head mode to all barrels
5.1.1. task.Headmode="1,2,8,"+CartridgeColumns;
5.1.2. This script can be used in "Advanced Settings" in conjunction with "Define Variables" to set the number of "Cartridge Columns" to the appropriate number.
5.2. Loop 3 times changing tips every 1 time.
5.2.1. Number of times to loop =NumberOfCupWashes
5.3. AM Aspirate 50uL from Position 2. (Utility Buffer)
5.3.1. Liquid class = CF_200uLperMin_10SecDelay
5.3.2. Distance from well bottom = 2
5.4. AM Dispense contents of tips to Position 9. (RP-S Cartridges)
5.4.1. Liquid class = CF_200uLperMin_10SecDelay
5.4.2. DIstance from well bottom = -13
5.5. AM Aspirate 75uL from Position 9. (RP-S Cartridges)
5.5.1. Liquid class = CF_200uLperMin_10SecDelay
5.5.2. Distance from well bottom = -17
5.6. AM Cartridges off at Position 9.
5.7. Dispense to waste contents of tips to Position 1. (Wash Station)
5.7.1. Distance from well bottom = 25
5.8. Wash tips with 240uL at Position 1. (Wash Station)
5.8.1. Liquid class = AM_50uLperSec
5.8.2. Mix cycles = 1
5.8.3. Distance from well bottom = 20
5.9. Move above location 1.
5.10. Dispense to waste contents of tips to Position 1. (Wash Station)
5.10.1. Distance from well bottom = 25
5.11. Loop End.

6. Internal Cartridge Washing
6.1. Set head mode to all barrels
6.1.1. task.Headmode="1,2,8,"+CartridgeColumns;
6.1.2. This script can be used in "Advanced Settings" in conjunction with "Define Variables" to set the number of "Cartridge Columns" to the appropriate number.
6.2. Loop 3 times changing tips every 1 time.
6.2.1. Number of times to loop = WashLoops
6.3. AM Aspirate 50uL from Position 2. (Utility Buffer)
6.3.1. Volume =WashVolume
6.3.2. Liquid class = CF_200uLperMin_10SecDelay
6.3.3. Aspirate flow rate =ElutionRate
6.3.4. Distance from well bottom = 2
6.4. AM Cartridges on from Position 9.
6.5. AM Dispense contents of tips to Position 4. (Flow Through)
6.5.1. Liquid class = AM_10uLperSec

6.5.2. Dispense flow rate = ElutionRate
6.5.3. Distance from well bottom = 2
6.6. AM Cartridges off at Position 9.
6.7. Loop End.
6.8. Wash tips with 240uL at Position 1. (Wash Station)
6.8.1. Liquid class = AM_50uLperSec
6.8.2. Mix cycles = 1
6.8.3. Distance from well bottom = 20
6.9. Move above location 1.
6.10. Dispense to waste contents of tips to Position 1. (Wash Station)
6.10.1. Distance from well bottom = 25

7. Stringent Syringe Washing
7.1. Set head mode to all barrels
7.1.1. task.Headmode="1,2,8,"+CartridgeColumns;
7.1.2. This script can be used in "Advanced Settings" in conjunction with "Define Variables" to set the number of "Cartridge Columns" to the appropriate number.
7.2. Loop 3 times changing tips every 1 time.
7.2.1. Number of times to loop =NumberOfCupWashes
7.3. AM Aspirate 50uL from Position 3. (Elution Buffer)
7.3.1. Liquid class = CF_200uLperMin_10SecDelay
7.3.2. Distance from well bottom = 2
7.4. AM Dispense contents of tips to Position 8. (Organic Waste)
7.4.1. Liquid class = CF_200uLperMin_10SecDelay
7.4.2. Distance from well bottom = 7
7.5. Wash Tips with 0uL at Position 1. (Wash Station)
7.5.1. Liquid class = AM_10uLperSec
7.5.2. Mix cycles = 0
7.5.3. Distance from well bottom = 20
7.6. Move above location 1.
7.7. Dispense to waste contents of tips to Position 1. (Wash Station)
7.7.1. Distance from well bottom = 25
7.8. Loop End.

8. Elution
8.1. Set head mode to all barrels
8.1.1. task.Headmode="1,2,8,"+CartridgeColumns;
8.1.2. This script can be used in "Advanced Settings" in conjunction with "Define Variables" to set the number of "Cartridge Columns" to the appropriate number.
8.2. AM Aspirate 100uL from Position 3. (Elution Buffer)
8.2.1. Volume =ElutionVolume
8.2.2. Liquid class = CF_200uLperMin_10SecDelay
8.2.3. Distance from well bottom = 1
8.3. Wash tips with 0uL at Position 1. (Wash Station)
8.3.1. Liquid class = AM_10uLperSec
8.3.2. Distance from well bottom = 20
8.4. AM Cartridges on at Position 9.
8.5. AM Dispense contents of tips to Position 7. (Eluate)

8.5.1. Liquid class = AM_10uLperSec
8.5.2. Dispense flow rate =ElutionRate
8.5.3. DIstance from well bottom = 2
8.6. Wash tips with 0uL at Position 1. (Wash Station)
8.6.1. Liquid class = AM_2_5sec Delay
8.6.2. Mix cycles = 0
8.6.3. Distance from well bottom = 22
8.7. AM Cartridges off at Position 9.
8.8. Wash tips with 240uL at Position 1. (Wash Station)
8.8.1. Liquid class = AM_50uLperSec
8.8.2. Mix cycles = 1
8.8.3. Distance from well bottom = 20
8.9. Move above location 1.
8.10. Dispense to waste contents of tips at Position 1. (Wash Station)
8.10.1. Distance from well bottom = 25