

PROTOCOL: IMAC Cartridge Preparation (TIP)

Purpose

Remove shipping solution, strip iron charged resin packed in AssayMap Bravo cartridges, and recharge them with iron chloride.

NB: This protocol should be run on the same day that the IMAC cartridges will be used. Ideally, the protocol should be executed immediately before the **IMAC** enrichment protocol.

Preparation

1. Prepare TIPMIX01 (100mM EDTA)
2. Prepare TIPMIX02 (100mM FeCl₃)
3. Prepare TIPMIX03 (0.1% TFA)

Materials

- HPLC-grade water, JT Baker, Cat. No. 4218-03 {TIP-M01}
- 500mM EDTA, Sigma-Aldrich, Cat. No. E7889-100ML {TIP-M02}
- FeCl₃, Sigma-Aldrich, Cat. No. 451649-1G {TIP-M03}
- Fe-NTA Agarose AssayMap Bravo Cartridges, Agilent Technologies {TIP-M04}
- Trifluoroacetic acid (TFA), Sigma-Aldrich, Cat. No. T6508-25ML {TIP-M05}
- 500uL V-bottom plate, VWR, Cat. No. 89005-016 {TIP-M06}
- 96-Well Half-Area Flat Bottom Microplate, Greiner Bio-One, Cat. No. 675101 {TIP-M07}
- 1-Well Low Profile Reagent Reservoir, Axygen, Cat. No. RES-SW1-LP {TIP-M08}

Assets

- Agilent AssayMap-BRAVO Automated Liquid Handling Platform with VWorks 4 {TIPA01}

Reagent Mixes

| ID | Name | Step | Composition | Volume/Well | Use |
|----------|-------------------------|------------|---|-------------|---|
| TIPMIX01 | 100mM EDTA | TIP | 1:5 dilution of 500mM EDTA {TIPM02} in HPLC-grade water {TIPM01} | 100 µL | Strips nickel from Ni-NTA charged agarose. |
| TIPMIX02 | 100mM FeCl ₃ | TIP | 16.2mg/mL FeCl ₃ {TIPM03} in HPLC-grade water {TIPM01} | 100 µL | Charges stripped NTA agarose cartridges with Fe ³⁺ ; CAUTION HIGHLY ACIDIC |

| | | | | | |
|----------|----------|------------|--|-----|--|
| TIPMIX03 | 0.1% TFA | TIP | 0.1% TFA {TIPM05} in HPLC grade water {TIPM01} | N/A | To wash internal and external AssayMap parts during protocol; CAUTION ACID |
|----------|----------|------------|--|-----|--|

Mix Preps and Mini-worksheets:

TIPMIX01 – 100mM EDTA (15mL)

1. Pipette 12mL of HPLC-grade water {TIP-M01} into a conical vial.
2. Pipette 3mL of 500mM EDTA {TIP-M02} into the vial.

TIPMIX02 – 100mM FeCl₃ (15mL)

1. Weigh out at least 243mg of iron chloride {TIP-M03} to make at least 15 mL
2. Calculate amount of water to add in mL by dividing amount weighed out by 16.2

- Amount weighed: _____ mg
- Divide by: 16.2
- Water to add _____ mL

TIPMIX03 – 0.1% TFA

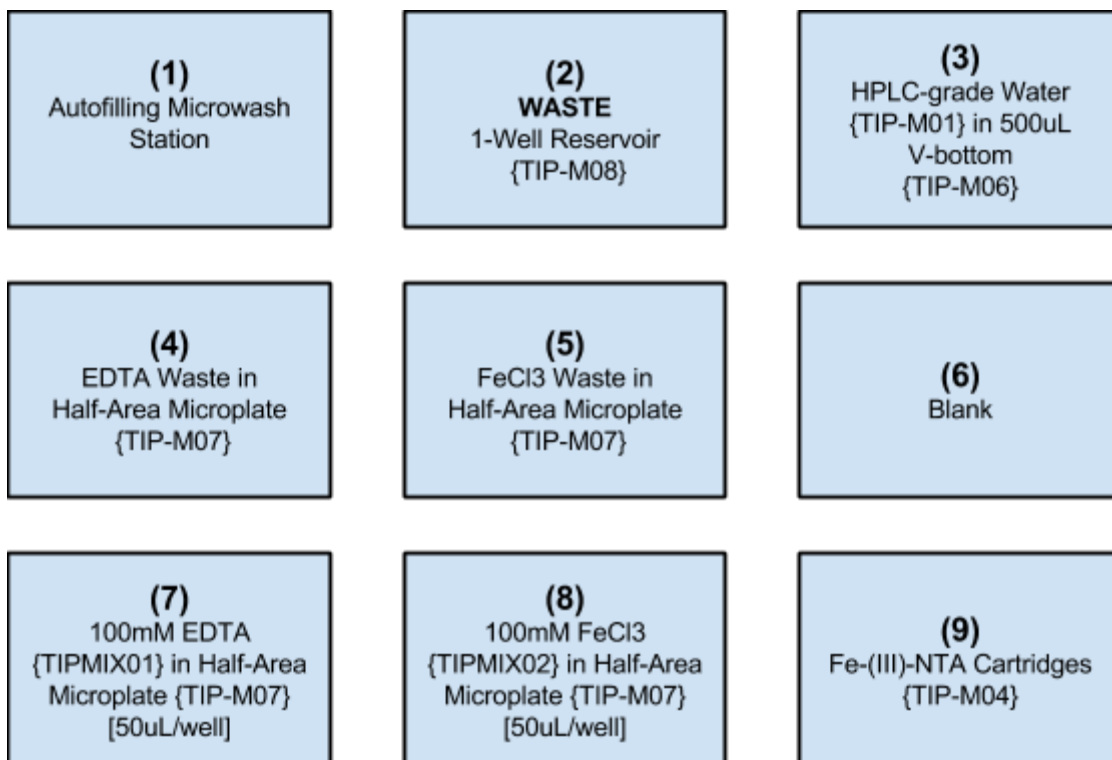
3. Pipette 999 mL of HPLC-grade water {TIP-M01} into a 1L bottle.
4. Pipette 1mL of TFA {TIP-M05} into the bottle.

Procedure

1. Aliquot 100uL of 100mM EDTA {TIPMIX01} into each well of a 96-well half-area flat bottom microplate {TIP-M07} with a multichannel pipette.
2. Aliquot 100uL of 100mM FeCl₃ {TIPMIX02} into each well of a 96-well half-area flat bottom microplate {TIP-M07} with a multichannel pipette.

NB: 100mM FeCl₃ is very acidic and should be handled with care. Waste FeCl₃ needs to be recovered from the microplate at the end of the protocol and disposed of in the appropriate waste container found in the chemical hood.

3. Fill a 500uL V-bottom plate {TIP-M06} with 300uL [requires 225uL] of HPLC-grade water {TIP-M01}.
4. Prepare AM-BRAVO for operation:
 - 4.1. Fill the Wash Station reservoir with 0.1% TFA 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.
 - 4.2. On the AM-BRAVO Eva {TIP-A01} load the device file "AssayMap Bravo_CF.dev". This file is located at C:\\VWorks Workspace\\Device Files\\.
 - 4.3. In the "Devices" page, click on "Agilent Bravo" and then "AM-EVA". Select "Initialize all devices".
 - 4.4. Open the protocol file "1_pSTY_AgilentIMAC_Tip_Prep_CF_V2.pro". This file is located at C:\\VWorks Workspace\\Protocol Files\\AM-Bravo\\IMAC\\.
5. Assemble the deck of the AM-BRAVO according to the following layout:



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. (TIP 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. (There should be no errors when running this simulation).
7. On the AM-BRAVO, toggle back to “Simulation is off”. Follow steps 6.1 to 6.3 in order to run the protocol. (TIP Automation Protocol Steps 2-6).
8. When the protocol is finished, do the following:
 - 8.1. Carefully check the IMAC cartridges for “iron charging”- they should have a faint yellow tint.
 - 8.2. Label IMAC tips located at the four corner positions with A1, A12, H1 and H12.
 - 8.3. Keep the IMAC cartridges on the deck or place at 4°C if not continuing immediately on to **IMAC Protocol**.
 - 8.4. Recover all FeCl₃ and dispose in the appropriate waste container in the chemical hood.
 - 8.5. Dispose of waste in the “Waste” reservoir at the appropriate satellite accumulation station.
 - 8.6. Retain any labware that can be reused. Empty appropriately, rinse with water, and leave to dry.

TIP Automation Steps (BRAVO-AssayMAP)

1. Define Variables
 - 1.1. CartridgeColumns = 3
 - 1.2. PrepVolume = 25
 - 1.3. SampleRate = 0.033
 - 1.4. WWV = 25
 - 1.5. WashRate = 0.417
2. Water Wash 1
 - 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 3 (Wash Station).
 - 2.2.1. Liquid class = AM_50uLperSec
 - 2.2.2. Mix cycles = 1
 - 2.3. Loop 3 times changing tips every 1 time.
 - 2.4. AM Aspirate 25uL from Position 3. (Water)
 - 2.4.1. Volume = WWV
 - 2.4.2. Distance from well bottom = 2
 - 2.5. AM Cartridges on from Position 9.
 - 2.6. Dispense to waste contents of tips to Position 1. (Wash Station)
 - 2.6.1. Liquid class = !AM_25uLperMin_0.42uLperSec
 - 2.6.2. Dispense flow rate =WashRate
 - 2.6.3. Distance from well bottom = 22
 - 2.7. AM Cartridges off at Position 9.
 - 2.8. Loop End.
3. Strip
 - 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. Loop 2 times changing tips every 1 time.
 - 3.3. AM Aspirate 25uL from Postion 7. (EDTA)
 - 3.3.1. Volume = PrepVolume
 - 3.3.2. Distance from well bottom = 0.5
 - 3.4. AM Cartridges on from Position 9.
 - 3.5. AM Dispense contents of tips to Position 4.
 - 3.5.1. Liquid class = AM_10uLperSec
 - 3.5.2. Dispense flow rate = SampleRate
 - 3.5.3. Distance from well bottom = 2
 - 3.6. AM Cartridges off at Position 9.
 - 3.7. Loop End.
 - 3.8. Wash Tips with 240uL at Position 3. (Wash Station)
 - 3.8.1. Liquid class = AM_50uLperSec

- 3.8.2. Mix cycles = 3
 - 3.9. AM Dispense contents of tips to Position 1. (Wash Station)
4. Water Wash 2
 - 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. Wash Tips with 240uL at Position 1 (Wash Station).
 - 4.2.1. Liquid class = AM_50uLperSec
 - 4.2.2. Mix cycles = 1
 - 4.3. Loop 3 times changing tips every 1 time.
 - 4.4. AM Aspirate 25uL from Position 3. (Water)
 - 4.4.1. Volume = WWV
 - 4.4.2. Distance from well bottom = 2
 - 4.5. AM Cartridges on from Position 9.
 - 4.6. Dispense to waste contents of tips to Position 1. (Wash Station)
 - 4.6.1. Liquid class = !AM_25uLperMin_0.42uLperSec
 - 4.6.2. Dispense flow rate =WashRate
 - 4.6.3. Distance from well bottom = 22
 - 4.7. AM Cartridges off at Position 9.
 - 4.8. Loop End.
5. Reload
 - 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 2 times changing tips every 1 times
 - 5.3. AM Aspirate 25uL from Positon 8. (FeCl3)
 - 5.3.1. Volume = PrepVolume
 - 5.3.2. Distance from well bottom =0.5
 - 5.4. AM Cartridges on at Position 9.
 - 5.5. AM Dispense contents of tips to Position 5.
 - 5.5.1. Liquid class = AM_10uLperSec
 - 5.5.2. Dispense flow rate = SampleRate
 - 5.5.3. Distance from well bottom = 2
 - 5.6. AM Cartridges off at Position 9.
 - 5.7. Loop End.
 - 5.8. Wash Tips with 240uL at Position 1. (Wash Station)
 - 5.8.1. Liquid class = AM_50uLperSec
 - 5.8.2. Mix cycles = 3
 - 5.9. AM Dispense contents of tips to Position 1.
6. Water Wash 3
 - 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. Wash Tips with 240uL at Position 3 (Wash Station).
 - 6.2.1. Liquid class = AM_50uLperSec

- 6.2.2. Mix cycles = 1
- 6.3. Loop 3 times changing tips every 1 time.
- 6.4. AM Aspirate 25uL from Position 3. (Water)
 - 6.4.1. Volume = WWV
 - 6.4.2. Distance from well bottom = 2
- 6.5. AM Cartridges on from Position 9.
- 6.6. Dispense to waste contents of tips to Position 1. (Wash Station)
 - 6.6.1. Liquid class = !AM_25uLperMin_0.42uLperSec
 - 6.6.2. Dispense flow rate =WashRate
 - 6.6.3. Distance from well bottom = 22
- 6.7. AM Cartridges off at Position 9.
- 6.8. Loop End.