PROTOCOL: Automated Protein Assay (APA)

Purpose

This assay quantifies protein amounts in P100 lysates for subsequent protein-level normalization.

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OVERVIEW:
Most pipetting steps occur on an Agilent Bravo LT liquid handler.  There are four
basic steps:
 1.   Creation of standard curve stocks (by hand).
 2.   Creation of standard curve assay plate (automated).
 3.   Creation of assay plates (automated).
 4.   Readout of standard and assay plates using a spectrophotometer.
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Preparation

1. If **LYSATE** plate is frozen, thaw it with Thaw Station {APAA03}.
    1.1. Remix each tube with brief vortexing.
    1.2. Spin at 15,000 x **g** for 15 min at 15 ‘C.

Materials

- P100 Lysis Buffer {APAM01} - from LYSIS protocol
- BSA protein standard 2 mg/mL, Thermo Scientific, Cat. No. 23210 {APAM02}
- A660 protein assay reagent, Thermo Scientific, Cat. No. 22660 {APAM03}
- HPLC-grade water, JT Baker, Cat. No. 4218-03 {APAM04}
- **LYSATE** plate (Matrix 96 tube rack, Thermo Scientific, Cat. No. 4274) {APAM05}
- 15 mL conical tube, Falcon, Cat. No. 352097 {APAM06}
- 1.5 mL microcentrifuge tubes, Axygen, Cat. No. MCT175LC {APAM07}
- 2 x v-bottom 96-well PCR plate, Bio-rad, Cat. No. MSP9601 {APAM08}
- 2 x 1-well reagent holder plate, Axygen, Cat. No. RES-SW1-LP {APAM09}
- 5 x flat-bottom clear 96-well plate, Greiner Bio-One, Cat. No. 655101 {APAM10}
- 4 x bravo 180F uL 96-tip racks*, Agilent Technologies, Cat. No. 19477-042 {APAM11}
  * in some steps only partial racks are consumed

Assets

- Agilent Bravo LT with VWorks4 {APAA01}
- SpectraMAX Spectrophotometer {APAA02}
- BioMicroLab Rack Thawing Station {APAA03}

Reagent Mixes

Standard Curve Diluent (prepared in step 1) {APAMIX01}

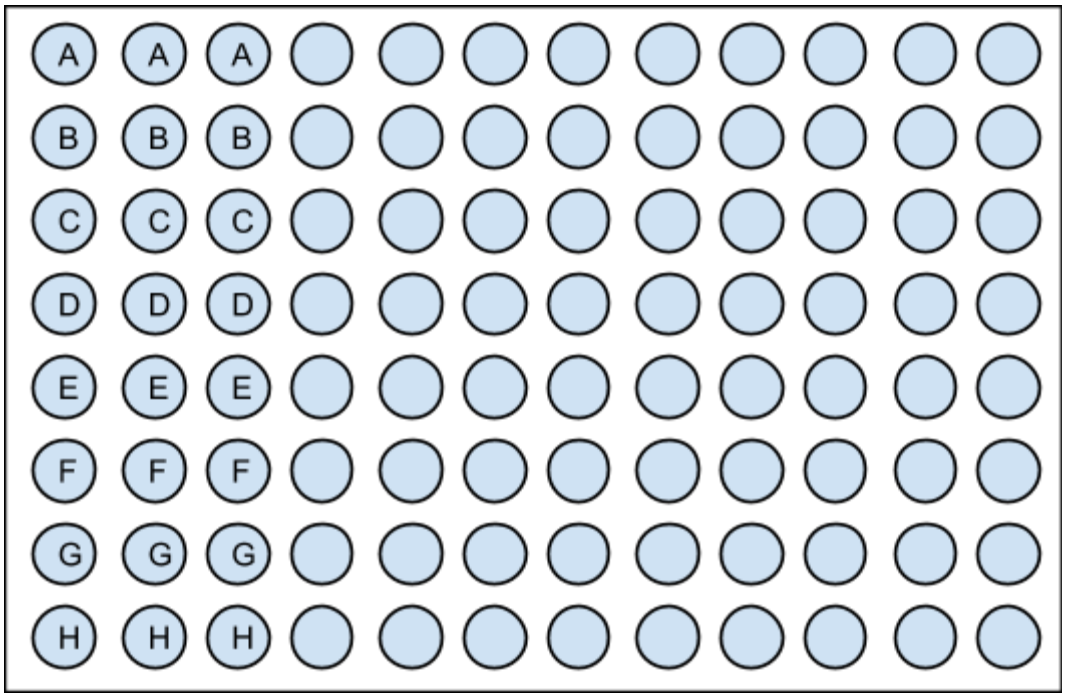
Procedure

NB:
The samples are assayed at a 1/5x dilution. Therefore the standard curve is constructed using 1/5x lysis buffer as background.

1. Mix 1 mL of P100 lysis buffer {APAM01} with 4 mL of water {APAM04} in a conical tube. This constitutes APAMIX01.
2. Pipette 200 uL of APAMIX01 into each of 8 x 1.5 mL microcentrifuge tubes, labeled A-H.
3. Construct the standard curve as follows in the order below, mixing by vortex after each serial dilution:

Label	Level	Source Tube	Source Amount
A	1000 ug/mL	BSA protein standard 2 mg/mL {APAM02}	200 uL
B	500 ug/mL	A	200 uL
C	250 ug/mL	B	200 uL
D	125 ug/mL	C	200 uL
E	62.5 ug/mL	D	200 uL
F	31.25 ug/mL	E	200 uL
G	Blank	n/a	n/a
H	Blank	n/a	n/a

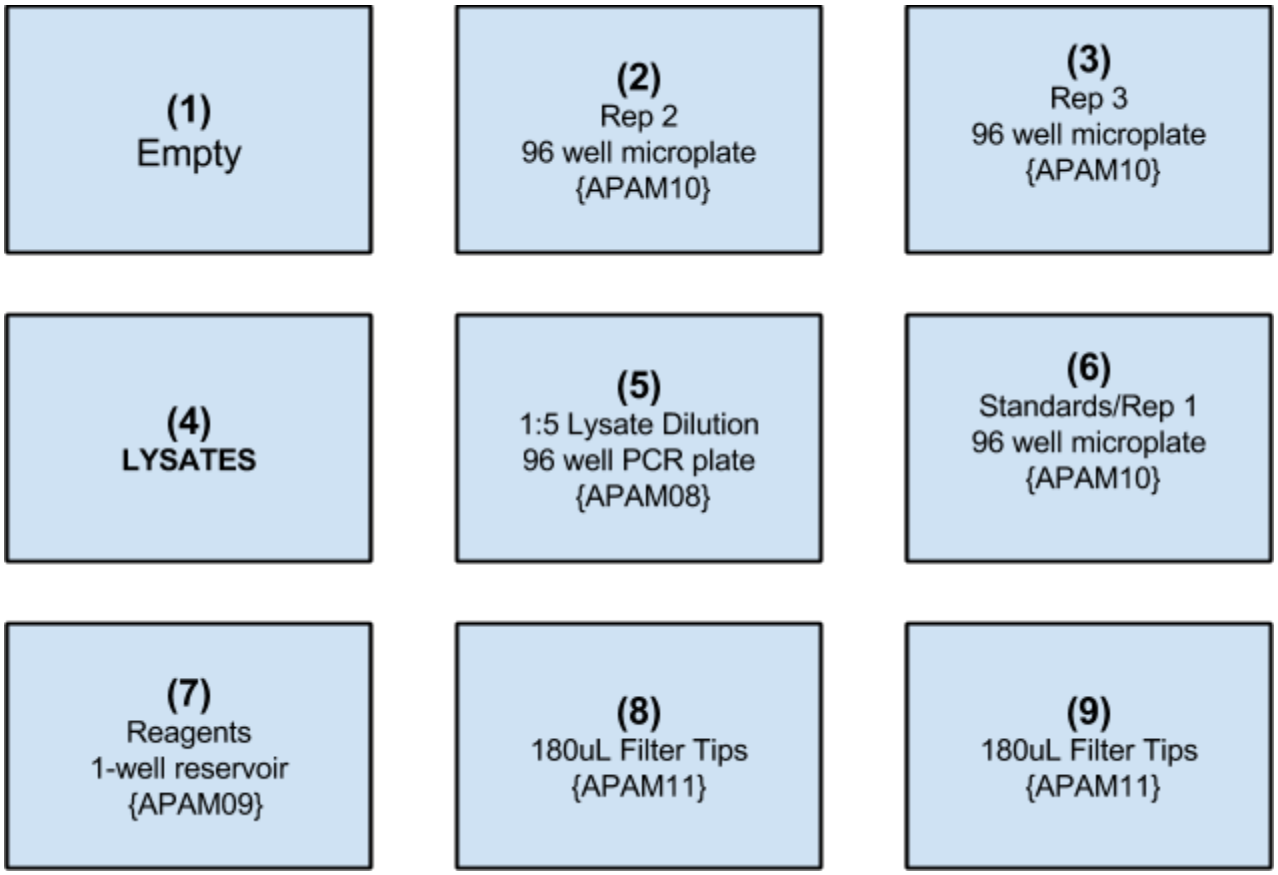
4. Pipette 10 ul of the contents of tubes A-H into each of 3 wells of a flat-bottom clear 96-well plate {APAM10} as shown:



STANDARD PLATE

5. Prepare LT-BRAVO for operation:
    5.1. On the LT-BRAVO Eva {APA-A01} load the device file “96LT_Bravo.dev”. This file is located at C:\\VWorks Workspace\Device Files\.
    5.2. In the “Devices” page, click on “Agilent Bravo” and then “96LT Bravo”. Select “Initialize all devices”.
    5.3. Open the protocol file “ProteinQuant_180F_Tips.pro”. This file is located at C:\\VWorks Workspace\Protocol Files\LT_Bravo\CF\P100\.
6. Assemble the deck with the following layout:

NB: The **Reagents** at position 7 should start the protocol with HPLC-grade water, this will be swapped out for A660 reagent mid-protocol. Position 6 should begin the protocol with the **Standards** plate which will be swapped out for **Rep 1** after the first addition of A660 reagent. The protocol will pause and prompt the user to make these changes.



8. Double check that all plates are squarely and securely seated in their respective positions.
9. Gently mix the bottle of A660 protein assay reagent {APAM03} by swirling.
10. Fill the reagent holder in position 7 with 70 mL of HPLC-grade water {APAM04} using a serological pipette.

NB: Have at the ready a 1-well reagent holder plate {APAM09} filled with 70 mL of A660 protein assay reagent {APAM03} and an additional rack of bravo 180F uL 96-tips {APAM11}. Some plates will be exchanged on the Bravo deck mid-protocol.

11. On the LT-BRAVO, toggle to “Simulation is on” at the top of the screen from “Simulation is off”.
    11.1. Press Start and the Run Configuration Wizard will pop up. Press Finish.
    11.2. A pop up entitled “Set Initial Values for Variables” will appear. Set the number of “Columns” to the appropriate amount of sample columns.
    11.3. Change the values for the other parameters listed if necessary and press ok.
    11.4. 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 LT-BRAVO user and obtain help.
12. On the LT-BRAVO, toggle back to “Simulation is off”. Follow steps 11.1 to 11.4 in order to run the protocol.

NB: The first segment of the protocol creates dilutions of the lysates for subsequent assay.

13. Watch the screen of the attached computer for the prompt to swap plates. When it appears:
    13.1. Swap the reagent holder in position 7 with 70 mL of A660 protein assay reagent {APAM03}.
    13.2. Swap the Bravo tips in position 8 for a fresh box of Bravo tips 180F uL {APAM11}.
14. Watch the screen of the attached computer for the prompt to swap plates. When it appears:
    14.1. Swap the Standards plate at position 6 with the Rep 1 plate.
15. Watch the screen of the attached computer for the prompt to swap plates. When it appears:
    15.1. Swap the reagent holder in position 7 with 70 mL of HPLC-grade water {APAM04}.
16. Clear the deck and proceed to **Protein-Level Normalization (DIL)**.

<ADD STEPS TO READ>