Host Protein Assay Protocol

Materials and Reagents Needed:

* Bovine Albumin Serum (BSA) Stock 2 mg/mL
* 96-well microplate -- the flimsy costar ones that are not lidded
* Bradford Reagent
* 1.5 mL conical tube
* Plate Reader
* Micropipettes with Tips

Step 1: Preparation

* Remove the host samples you wish to test from cold storage to thaw.
  + You can run up to 24 host samples per plate
  + Make sure you have at least 20µL per sample you wish to run
* Remove the BSA Stock aliquots from cold storage to thaw
* Prepare station with a fresh 96-well plate, reservoirs, and properly stocked micropipette tips

Step 2: Standard Curve

* Prepare a 600µL aliquot of BSA Stock at a concentration of 0.1µg/µL
  + Flick-and-spin BSA Stock aliquot to ensure it is properly mixed
  + Using the p-1000 pipette to add 570µL of seawater to 1.5 ml tube
  + Using the p-200 Pipette to add 30µL of BSA
  + Flick-and-spin vial to thoroughly mix solution
* From the 600µL stock, pipette 180µL of 0.1µg/µL BSA solution into first 3 wells of 96-well plate (A1, B1, and C1)
* Fill multichannel reservoir with sea water and load 7 pipette tips onto the p-200 multichannel micropipette
* Pre-wet tips and load the 7 wells under the 0.1 ug/UL wells with 80µL of seawater (A2-8, B2-8, and C2-8)
  + Remember to check to make sure multichannel is drawing up equal amounts of fluid in each tip
  + No need to change tips during this step
* Load the p-200 multichannel micropipette with 3 tips to 100µL and draw up 100µL of fluid from the first row of wells (A1, B1, and C1)
* Serially dilute the next 6 wells by pipetting up and down 5 times in each of the next wells (A2-7, B2-7, and C2-7)
  + Never go to the second stop
  + Do not pipette in the last well (A8, B8, and C8)
  + And discard fluid in tip after the 7th well
* Check to make sure all wells have an equal volume of liquid (equal to 80µL)

Step 3: Load Samples

* Plan your plate layout so it is clear and easy to understand allowing 3 wells per sample (each sample will be done in triplicate)
* Using the p-200 multichannel pipette, load each sample well with 73.6µL of seawater
  + Make sure to pre-wet tips
* Run samples in micro-centrifuge for 2 minutes at 3,500 rpm to settle particulates
* Using the p-20 micropipette and drawing from center of tube, load 6.4µL of sample per well, keeping track of locations in lab notebook
  + Take care not to disturb the pellet
  + Make sure entire volume is expelled from pipette by back pipetting 3 times in well

Step 3: Addition of Bradford

* Remove Bradford Reagent aliquot from fridge and add a sufficient amount to reservoir
* Using p-200 multichannel and p-200 as necessary add 80µL of Bradford to each well that contains fluid, back pipetting 5 times to thoroughly mix
  + Use a clean set of tips in every new column
  + Make sure tips are pre-wetted
  + Try to add the Bradford reagent as quickly as possible
  + Avoid creating bubbles in well
  + Check to make sure all wells have a roughly equal amount of volume (equal to 160µL)
* Cover with a kimwipe or plate cover to help slow evaporation of liquid
* Let incubate at room temperature for 10-20 minutes
  + Use timer to keep track

Step 4: Machine Set Up

* While the plate is incubating, open the laptop at the microplate reader and login.
* Launch the Gen5 program
* Set up protocol as follows (also accessible under Protocol labeled as “Procedure”)
  + Hit New..
  + Click Shake
    - Shake Mode: Orbital
    - Duration: set to 0:05
    - Orbital Frequency: 282cpm (3mm)
    - Orbital Speed: Slow
    - Hit OK
  + Click Read
    - Detection Method: Absorbance
    - Read Type: Endpoint/Kinetic
    - Optics Type: Monochromators
    - Click OK
    - Under Wavelengths change 450 to 595
    - Read Speed: Normal
    - Pathlength Correction: Unchecked
    - Click OK
  + Click OK
  + Press Cancel if the Plate is Asking to be read
* Click Protocol
  + In drop-down menu select “Plate Layout”
  + Select the boxes labeled Blanks, Standard Curves, and Samples
  + Click Next >
  + In “Blank” menu
  + In menu called Standard Curve #1
    - Change Replicates: to 3
    - Set Units: to µg/µL
    - In the “Auto” box select the Ration box and input in the adjacent box 1.8
    - Make sure Type is set to Concentrations
    - In box labeled STD1 input 0.05
    - Hit down arrow 6 times so the boxes up through STD7 are filled in
    - Select Next >
    - In menu labeled “Sample” hit the Finish button
* In next window
  + Select BLK from side menu and select box H1, H2, and H3
  + Click 0.05 under STD
  + Click box A1 and drag down to G1
  + Repeat for A2-G2 and A3-G3
  + Click SPL1 under Sample and highlight any box that correlates to a well that contains a Sample
  + Select OK

Step 5: Read Plate

* After the 10-20 minute incubation period place the well plate in microplate reader
  + If tray is not already open press black button next to the power toggle on the machine
* Orient tray so that well A1 is in the right corner closest to the machine (check for A1 mark on the carousel)
* Once the plate is on the carrier press the circular green button in the Gen5 software with the white ▶️ (PLAY) logo
* Press OK
* The machine will begin reading the plate
* Save your data!

Step 6: Clean Up

* Clean up plate properly disposing of all chemicals in proper receptacle

Standard

|  |  |
| --- | --- |
| Standard # | Concentration (µg/µL) |
| 1 | 0.1 |
| 2 | 0.05555555556 |
| 3 | 0.03086419753 |
| 4 | 0.01714677641 |
| 5 | 0.009525986892 |
| 6 | 0.00529221494 |
| 7 | 0.002940119411 |

Plate Layout:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | STD1 | STD1 | STD1 | SPL1 | SPL1 | SPL1 | SPL9 | SPL9 | SPL9 | SPL17 | SPL17 | SPL17 |
| B | STD2 | STD2 | STD2 | SPL2 | SPL2 | SPL2 | SPL10 | SPL10 | SPL10 | SPL18 | SPL18 | SPL18 |
| C | STD3 | STD3 | STD3 | SPL3 | SPL3 | SPL3 | SPL11 | SPL11 | SPL11 | SPL19 | SPL19 | SPL19 |
| D | STD4 | STD4 | STD4 | SPL4 | SPL4 | SPL4 | SPL12 | SPL12 | SPL12 | SPL20 | SPL20 | SPL20 |
| E | STD5 | STD5 | STD5 | SPL5 | SPL5 | SPL5 | SPL13 | SPL13 | SPL13 | SPL21 | SPL21 | SPL21 |
| F | STD6 | STD6 | STD6 | SPL6 | SPL6 | SPL6 | SPL14 | SPL14 | SPL14 | SPL22 | SPL22 | SPL22 |
| G | STD7 | STD7 | STD7 | SPL7 | SPL7 | SPL7 | SPL15 | SPL15 | SPL15 | SPL23 | SPL23 | SPL23 |
| H | BLK | BLK | BLK | SPL8 | SPL8 | SPL8 | SPL16 | SPL16 | SPL16 | SPL24 | SPL24 | SPL24 |