Semi-Automated Western Blot

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1. Overview

The purpose of this protocol is to standardize procedures for accomplishing a 96 well plate based semi-automated western blot procedure utilizing the Integra Viaflow for sample washing and lysis, and the Integra Assist Plus for sample normalization.

Materials Required

- Micropipettes (P20, P200, P1000)
- 96 well deep well plates
- 96 well round bottom plates (Falcon 305077)
- Antibodies of interest
- Bio-Rad standard western materials (Acrylamide gel, membranes, protein ladder etc.)

Safety Considerations

Wear appropriate PPE including gloves, lab coat, and safety glasses. Prepare and add sample buffer with Beta-mercaptoethanol under a fume hood.

2. Procedure

Day 0

1.
   - Plate cells at 500K cells/well in a 12 well culture plate in 1 mL of culture media.
   - Suspension cells can be dosed this same day. (Skip Day 2 of the procedure in this case).
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Day 1

- Dose compounds for adherent cells.

Day 2

- For suspension cells, collect total 1 mL volume per well into 96 well deep well plate and centrifuge for 5 minutes at 300 G.
- For adherent cells aspirate culture media, wash with PBS, and proceed to add 300 uL of Trypsin to detach cells from plate before adding 700 uL of culture media and collecting 1 mL total volume per well into 96 well deep well plate and centrifuging for 5 minutes at 300 G.
- Inspect the deep well plate from below to ensure cell pellets are visible and present. After inspection proceed to use the Integra Viaflow in the dark room for liquid aspiration by running protocol "COLSEN_WB". Prior to running this protocol ensure that the plate deck has been pushed as far to the right as possible and that a waste receptacle for collecting the aspirated liquid is set up in the center of the triple deck position. Place the deep well block containing samples in the leftmost deck position. Use the image below as a reference.
- Next, re-suspend cell pellets in 300 uL of PBS by manually operating the viaflow using the pipette and mix functionality. Centrifuge the deep well sample plate again for 5 minutes at 300 G.
- Aspirate the PBS by running the "COLSEN_WB_WASH" protocol and using the same deck setup as in the previously referenced image above.
- Prepare Cytobuster Lysis buffer with added HALT protease and Phosphatase inhibitors.
- Lyse the cells by manually operating the viaflow using the pipette and mix functionality.
- Transfer the lysate solution to a round bottom 96 well plate by running the "COLSEN_100uL_MOVE" protocol with the deep well plate on the left platform position and the round bottom 96 well plate on the right.
- Freeze samples in -80 C freezer

Day 3

- Thaw sample plate on ice.
- Centrifuge sample plate at maximum speed for 15 minutes with centrifuge set to 4 C.
- Transfer the protein solution to a new round bottom 96 well plate by running the "COLSEN_100uL_MOVE" protocol with the previous 96 well plate on the left platform position and the new round bottom 96 well plate on the right.
- Run the Bio-Rad DC Protein Assay according to the manufacturer's specifications manual
- Generate a worklist for Cytobuster normalization values and import it to D-ONE pipette using the "Immunoblot_Normalization_V1" protocol.
- Generate a worklist for protein normalization values and import it to D-ONE pipette using the "Immunoblot_Normalization_Add_Sample_V1" protocol. Check the pipetting parameters for this protocol before running it to ensure liquid level detection is configured not to prompt the user but to go to the designated height. Also, make sure mixing speeds/volumes are not too high and aspiration speed is not too high.
- Manually add sample buffer using a multichannel pipette.
- Put the plate on a heat block for 5 minutes at 95 C.
- Run the standard WB protocol from here on out.