STANDARD OPERATING PROCEDURE

Title: Protein Separation and Identification using Automated Western

SOP#: M-134

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1. PURPOSE

This procedure is to be used for protein separation and identification under automated conditions by incorporating Simple Western™ immunoassay technology on the Wes™ instrument by ProteinSimple.

2. SCOPE

This document describes the Simple Western™ immunoassay procedure using the Wes™ instrument. The Wes™ instrument allows protein separation by size or charge. Protein identification and quantitation are performed on the Wes™ instrument through total protein and immune detections and standard curve generation, respectively. Protein size separation and immune detection will be described in this SOP.

3. RESPONSIBILITIES

It is the responsibility of person(s) performing this procedure to be familiar with lab safety procedures and Compass software for Simple Western™ Assays. All procedural steps are to be followed as written and any deviations, problems and observations during an experiment must be documented. The interpretation of the results must be done by a person trained in the procedure and familiar with such interpretation.

4. EQUIPMENT

- Wes™ instrument; ProteinSimple
- Centrifuge with plate adaptor (Eppendorf, Model 5810R, 15amp with swing bucket rotor or equivalent)
- Standard Heat Block Heater (VWR, Catalog #12621-104, Model 949310 or equivalent)

- Vortex

5. MATERIALS

- Wes 12-230 kDa Rabbit or Mouse Master Kit w/Split Buffer for 104 data points; ProteinSimple, Cat. # PS-MK16 and PS-MK17, respectively.
- Protein Samples corresponding to the antibodies to be evaluated
- Primary Antibodies to be tested
- Water (molecular biology grade or better)
- 10X Phosphate Buffered Saline (PBS); Fisher Scientific, Cat. #BP399-1 (diluted to 1X with water)
- 5 X SignaLOCK™ Blocking Solutions; KPL, Cat. # 50-58-00 (diluted to 1X with water)
- Pipettes and tips
- Micro centrifuge tubes
- Ice and ice bucket

6. REAGENTS

6.1. For rabbit or mouse monoclonal antibody evaluation

- Protein corresponding to the antibody.
- Primary antibody corresponding to the protein
- Anti-rabbit or anti-mouse secondary antibody; ProteinSimple, Cat. # 042-206 or 042-205, respectively (comes with Master Kit).

7. PROCEDURE

7.1. Preparation of Standard Pack Reagents

7.1.1. Follow Wes 12-230 kDa Rabbit or Mouse Master Kit w/Split Buffer protocol.

7.2. Preparation of Samples (proteins)

7.2.1. Follow Wes 12-230 kDa Rabbit or Mouse Master Kit w/Split Buffer protocol with some modifications as noted below:

- Dilute samples (proteins) in 0.1X Sample Buffer (supplied 10x in kit) for an intermediate stock (IS) concentration of 0.00125 ug/uL.

- Instead of combining 1 part 5X Fluorescent Master Mix with 4 parts protein prep, combine 1 part 5X Fluorescent Master Mix with 5 parts IS protein prep in a micro centrifuge tube (final concentration 0.00104 ug/uL). Mix with gentle pipetting.

  Example: Add 2 uL 5X Fluorescent Master Mix to 10 uL IS protein prep (5.2 ng/5 uL per microplate well).

- Denature samples (proteins) and biotinylated ladder @ 95°C, 5 min. using the heat block heater. Then vortex, do a quick spin at Room Temperature and store on ice until ready to proceed.

7.3. Preparation of Antibodies

7.3.1. Follow Wes 12-230 kDa Rabbit or Mouse Master Kit instructions w/Split Buffer protocol with some modifications noted below:

- Instead of diluting primary antibodies in Antibody Diluent II, dilute primary antibodies in1X PBS for an IS concentration of 1 ug/mL.
- Then dilute IS 1:5 in 1X SignaLOCK™ Blocking Solution (final concentration 0.2 ug/mL).

Example: Add 20 uL IS antibody prep to 80 uL 1X SignaLOCK™ Blocking Solution (2 ng/10 uL per microplate well).

7.3.2. Secondary antibody is supplied with the kit and is ready to use without dilution.

7.4. Preparation of Luminol-S and Peroxide

7.4.1. Follow Wes 12-230 kDa Rabbit or Mouse Master Kit w/Split Buffer protocol.

7.5. Microplate Set -Up

7.5.1. Dispense reagents into microplate using the volumes shown in the plate diagram of the Wes 12-230 kDa Rabbit or Mouse

Master Kit w/Split Buffer protocol with some modifications noted below:

- Add 0.1X Sample Buffer into unused row A wells.
- Instead of adding 10 uL Antibody Diluent II into row B wells, add 10 uL Antibody Diluent II into B7 well and add 10 uL 1X SignaLOCK™ Blocking Solution into B8 – B19 wells.

7.5.2. Centrifuge microplate for 5 min, @ 2500 rpm (~1000 x *g*) at room temperature. Ensure liquid is fully down in all wells.

7.6. Starting Wes Assay

7.6.1. Load Wes-13 Size assay in Compass software on the Wes Instrument with some modifications noted below:

- Change Antibody Diluent Time from 5 min to 30 min.
- Change Detection Profile from 7 Exposures to 8 Exposures (1, 5, 15, 30, 60, 120, 240 and 480 seconds).

7.6.2. Label template with pertinent information such as sample names/concentration, Antibody names/concentration, Ladder name, etc.

7.6.3. Then Open Wes' door and follow Wes 12-230 kDa Rabbit or Mouse Master Kit w/Split Buffer protocol.

8. REFERENCED DOCUMENT

8.1. Operation Manual, Wes™ Instrument

8.2. Operation Manual, Compass Software

8.3. Master Kit protocol, Wes 12-230 kDa Rabbit or Mouse Master Kit w/Split Buffer for 104 data points.