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 Jess ™ instrument by ProteinSimple.

2. SCOPE

This document describes the Simple Western™ immunoassay procedure using the Jess™ instrument. The Jess™ instrument allows protein separation by size or charge. Protein size separation and immuno-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

- Jess™ instrument; ProteinSimple (Bio-Techne).
- 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

- Jess 12-230 kDa Separation Module; ProteinSimple, Cat. # SM W004. Note: separation module contains 13- or 25-Capillary Cartridge (SM W002 or SM W004), pre-filled microplates, wash buffer and 10X sample buffer. It also comes with EZ Standard Pack, 12-230 KDa, PS- ST01EZ-8
    - SM-004 is the most commonly used since most proteins are in the 12-230 kDa range The 2-40 kDa cartridge (SM-010) would be used for low molecular weight proteins and the 66-440 kDa cartridge (SM-006) would be used for high molecular weight proteins.
- Primary Antibodies to be tested
- Water (molecular biology grade)
- Pipettes and tips
- Micro-centrifuge tubes
- Ice and ice bucket

6. REAGENTS

6.1. For rabbit or mouse monoclonal antibody evaluation

- Protein samples (recombinant protein, over-expressed lysate, cell or tissue lysate) corresponding to the antibodies to be evaluated
- Primary antibody to be tested
- Anti-Rabbit or Anti-Mouse Detection Modules (respectively DM-001 and DM-002) containing Luminol-S (Cat. # 043-311), Peroxide (Cat. # 043-379), Streptavidin-HRP (Cat. # 042-414), Antibody Diluent #2 (Cat. # 042-203)
- EZ Standard Pack (included in separation module SM), containing Ready to use biotinylated ladder (12-230 kDa, Cat. # PS-ST01EZ), Fluorescence 5X Master Mix, and DTT.

7. PROCEDURE

7.1. Preparation of Standard Pack Reagents

7.1.1. Follow Separation Module (SM-W001 – SM-W012) insert indications to mix Fluorescent 5X Master Mix, ladder and DTT.

7.2. Preparation of Samples

7.2.1. Follow directions in Separation Module (SM W001 – SM W012) insert.

- Dilute samples (proteins) in 0.1X Sample Buffer (supplied 10x in kit) for an intermediate stock (IS) concentration of 0.250 ug/uL.
- Combine 1 part 5X Fluorescent Master Mix with 4 parts IS protein prep in a micro centrifuge tube (final concentration 0.2 ug/uL). Mix with gentle pipetting.

  Example: Add 2 uL 5X Fluorescent Master Mix to 8 uL IS protein prep

- 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 (Step 7.5.2.1).

7.3. Preparation of Antibodies

7.3.1. Dilute the antibody in antibody Diluent #2 to final concentration of 0.2 ug/mL, for a minimum final volume of 500 uL.

7.3.2. Secondary antibody (anti- mouse or anti-rabbit) is supplied with the kit and is ready to use without dilution.

7.4. Preparation of Luminol-S and Peroxide

7.4.1. Combine 200 uL of Luminol-S and 200 uL of Peroxide.

7.5. Microplate Set -Up

7.5.1 On the acquisition software (Compass^{TM}), from the Assay tab select File→ New Assay →Jess and indicate the immunoassay type (chemiluminescence), size range (typically 12-230 KDa), and cartridge size (typically 25).

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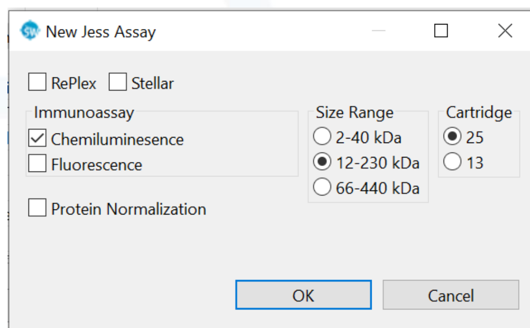
Example:



7.5.2 Use the Assay tab to define the plate layout (sample position on the plate and concentration)

7.5.2.1 Dispense reagents into microplate as defined above. **Note: this procedure diverges from the insert instructions for the sample volume, loading 5 uL of sample, rather than 3 uL.**

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

7.6. Starting Jess Assay

7.6.1. In the Assay tab (protocol tab) set up the assay:

- Set Antibody Diluent Time to 30 minutes
- Set Separation Time to 30 minutes

Example:

Protocol | History | Notes

	Value
› Separation Matrix	
› Stacking Matrix	
› Sample	
Separation Time (min)	30.0
› Separation Voltage (volts)	375
› Antibody Diluent Time (min)	30.0
› Primary Antibody Time (min)	30.0
› Secondary Antibody Time (min)	30.0
⌄ Detection	
Well Row	E1
Detection Profile	HDR

7.6.2. Then Open Jess' door and follow Jess separation module protocol.

8. REFERENCED DOCUMENT

8.1. Operation Manual, Jess^{TM} Instrument

8.2. Operation Manual, Compass^{TM} Software

8.3. Separation Module Protocol (SM-W001 - SM W012).