STANDARD OPERATING PROCEDURE

Title: Single-Cell Protein Separation and Identification using Single-Cell Western

SOP#: M-136

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1. PURPOSE

This procedure is to be used for single-cell protein separation and identification under automated conditions by incorporating Single-Cell Western™ assays on Milo™ instrument and fluorescence microarray scanner by ProteinSimple.

2. SCOPE

This document describes the Single-Cell Western assay and detection procedure using the Milo™ instrument and microarray scanner. The Milo™ instrument allows single-cell protein separation by size or charge on a scWest chip. Protein identification and quantitation are performed on a compatible fluorescence microarray scanner through imaging of fluorescently tagged secondary antibody followed by data automation via Scout Software. Single-cell protein size separation, identification and quantitation 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 Scout software package for Single-Cell Western™ Assays. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

4. EQUIPMENT

- Milo™ instrument; ProteinSimple, Cat. # P100
- InnoScan 710 microarray scanner (or equivalent); Innopsys but supplied by ProteinSimple
- Scout Software; https://www.proteinsimple.com/scout/downloads/

- Brightfield cell culture microscope capable of 10X magnification
- Standard Laboratory Cell Culture CO_2 Incubator
- Biosafety Cabinet/Hood Class II
- Computer for image analysis with appropriate software (i.e. scout software and scanner software) installed for this application and analysis
- Microarray slide spinner; Sigma Aldrich, Cat. # Z674664-1EA
- Probing Chamber and sponges; supplied with Milo, Cat. # A200
- Tweezers; supplied with Milo, Cat. # 035-023
- Centrifuges; Labnet, Cat. # C1601 and Marshall Scientific, Cat. # SO-LEGRT or equivalent
- Benchtop vortex; Benchmark Scientific, Model: Vornado, Cat. # BV101-G or equivalent
- Benchtop rocker; Benchmark Scientific, Model: Mini BlotBoy, Cat. #B3D1008 or equivalent

5. MATERIALS

- Standard scWest Kit; ProteinSimple, Cat. # K600
- Protein Samples (not peptides due to small MW) corresponding to the antibodies to be evaluated
- 0.05% Trypsin-EDTA (1X); Gibco, Cat. # 25300-062 or equivalent
- Primary Antibodies to be tested corresponding to the proteins of interest
- Water (Deionized or better)
- 500 ml beaker/bottles
- Pipettes and tips
- Non-treated petri dishes, D x H (150 mm x 25 mm); Sigma Aldrich, Cat. # CLS430597
- Non-treated petri dishes, D x H (100 mm x 20 mm); Sigma Aldrich, Cat. # CLS430591-100EA
- Micro centrifuge tubes, 15 mL
- 50 mL conical tubes
- Aluminum foil

6. REAGENTS

6.1. For rabbit or mouse monoclonal antibody evaluation

- Cells corresponding to the antibody or protein of interest such as cell suspensions, trypsinized adherent cell lines, frozen cells, cells isolated from tissues and dissociated tissues.
- Purified monoclonal antibody corresponding to the protein of interest
- Fluorescently labeled secondary antibodies such as Donkey anti-rabbit or Donkey anti-mouse NL557 secondary antibody; R&D System, Inc., Cat. # NL004 or NL007, respectively, or equivalent.

7. PROCEDURE

7.1. Preparation of 1X Suspension and Wash Buffers

7.1.1. Follow Standard scWest Kit protocol.

7.2. Preparation of scWest Chip

7.2.1. Follow Standard scWest Kit protocol.

7.3. Preparation of single-cell suspension

7.3.1. Follow Standard scWest Kit protocol and dilute cells to 100,000 cells/mL with 1X Suspension Buffer.

7.4. Load cells on scWest Chip

7.4.1. Follow Standard scWest Kit protocol with following recommendations:

- Place scWest chip in another Non-treated petri dish, D x H (100 mm x 20 mm) with gel side up and load 1mL cell suspension dropwise on top of scWest chip.
- Let cells settled for 15-20 minutes.
- Remove cells from slide by gently tapping scWest chip onto petri dish and then wash off unsettled cells with 1 ml 1X Suspension Buffer, 2X.

7.5. Run scWest Chip on Milo

7.5.1. Follow Standard scWest Kit protocol.

7.6. Probe scWest Chip with Primary and Secondary Antibodies

7.6.1. Follow Standard scWest Kit protocol with following recommendations:

- Before adding antibodies, place clean probing chamber(s) into a Non-treated petri dish, D x H (100 mm x 20 mm) for one scWest chip or into a Non-treated petri dish, D x H (150 mm x 25 mm) for two scWest chips.
- Dampen sponges with DI water and place two sponges per petri dish.
- After adding primary and secondary antibodies accordingly, place petri dish lid on top of dish and incubate accordingly.
- Wrap petri dish in foil during secondary antibody incubation and washes.

7.7. Image scWest Chip and Analyze Data with Scout Software

7.7.1. Follow Standard scWest Kit protocol and dry scWest chip in a microarray slide spinner for 5 minutes instead of 3 minutes as indicated in the ScWest Kit protocol.

8. REFERENCED DOCUMENTS

8.1. Operation Manual, Milo™ Instrument

8.2. Operation Manual, Scout Software

8.3. Standard scWest Kit Protocol

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