User Guide for Milo

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User Guide for Milo

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ProteinSimple's Single-Cell Western technology is covered by issued and pending patents in the U.S. and other countries. For more information please see www.proteinsimple.com/Intellectual_Property.html.

Table of Contents

Chapter 1:

Let's Get Started

Chapter Overview

- Welcome
- Single-Cell Western Assays on Milo
- Overview of Single-Cell Western Protocol

Welcome

Congratulations on bringing Milo into your lab! We welcome you as a new user and are excited to be a part of your work as you discover new biological insights and truly understand the uniqueness of each single-cell within your complex samples.

To help you get the most from Milo, we've added some attention phrases to guide you through the user guide:

NOTE	Points out useful information
IMPORTANT	Indicates information necessary for proper operation of Milo
CAUTION	Cautions you about potentially hazardous situations that could result in injury to you or damage to Milo
	Indicates information that can give you confidence that your assay is running correctly

Single-Cell Western Assays on Milo

This user guide will provide you with the information you need to partition cells from a single-cell suspension into individual microwells on a scWest chip and to perform Single-Cell Western (scWestern) analysis on isolated cells. The user guide explains all steps needed including: single-cell capture, cell imaging for confirmation of cell isolation, cell lysis, SDS-PAGE on individual cell lysates, photo-immobilization of separated protein bands, antibody probing, protein target imaging, and archiving of scWest chips.

A Single-Cell Western assay is a multiplexed western blot on ~1,000+ single-cells in parallel. Single-Cell Western assays measure protein expression in each analyzed single-cell.

Single-Cell Western assays take place on an scWest chip. Milo automates the key assay steps required to run an scWest chip including cell lysis, SDS-PAGE separation of each single-cell lysate and immobilization of separated protein bands. One sample is loaded per scWest chip which yields 1,000+ Single-Cell Westerns per chip.

The scWest chips described in this protocol use a modified photoactive polyacrylamide gel that covalently binds protein bands into the gel after electrophoretic separation, eliminating the protein transfer and immobilization step of conventional blotting procedures. This technique allows users to directly measure protein expression with single-cell resolution rather than having to rely on RNA-based measurements to study gene expression. The technique is compatible with commercially available antibodies and is well suited for analysis of intracellular protein targets that cannot be easily studied with flow cytometry. In addition to providing protein expression information at single-cell resolution to understand cell subpopulations, the Single-Cell Western workflow also saves time over conventional Western blotting techniques. The scWest chips can also be archived for future re-probing of precious cellular samples.

Overview of Single-Cell Western Protocol

Steps		Time
Cell and reagent preparation		
1.	Dilute 10X Suspension Buffer to 1X Suspension Buffer Rehydrate scWest chip in 1X Suspension Buffer	10+ minutes (may be performed during cell preparation)
2.	Dilute 5X Wash Buffer to 1X Wash Buffer	5 minutes
3.	Create single-cell suspension in 1X Suspension Buffer	15–30 minutes
4.	Dilute cells to 10,000-100,000 cells/mL in 1X Suspension Buffer	5 minutes
Cell loading and QC		
5.	Pipette cells onto scWest chip	1 minute
6.	Let cells settle	5–20 minutes
7.	Wash 1-3X with 1 mL 1X Suspension Buffer	5 minutes
8.	Inspect scWest chip on brightfield microscope to confirm cell isolation	5 minutes
Electrophoresis and photo-immobilization		
9.	Enter desired run parameters into Milo	1 minute
10.	Place scWest chip into Milo	1 minute
11.	Pour Lysis/Run Buffer into buffer reservoir	
12.	Close lid and run cell lysis, separation, and protein capture	5–6 minutes
Antibody probing		
13.	Remove chip and place in Petri dish; quick-wash with 15 mL of 1X Wash Buffer	1 minute
14.	Add fresh 1X Wash Buffer, wash on shaker	2x10 minutes
15.	Prepare primary antibody solution	5 minutes
16.	Add primary antibodies to scWest chip using probing chamber	1 minute
17.	Incubate primary antibodies	1–2 hours*
18.	Remove chip and place in Petri dish with 15 mL 1X Wash Buffer	1 minute
19.	Add fresh 1X Wash Buffer, wash on shaker	3x10 minutes

Overview of Single-Cell Western Protocol, *continued.*

Steps		Time
20.	Prepare secondary antibody solution	5 minutes
21.	Add secondary antibodies to scWest chip using probing chamber	1 minute
22.	Incubate secondary antibodies	30–60 minutes*
23.	Remove chip and place in Petri dish with 15 mL 1X Wash Buffer	1 minute
24.	Add fresh 1X Wash Buffer, wash on shaker	3x15 minutes
25.	Scan on microarray scanner	8–35 minutes
Total		**~4–6.5 hours**

*Antibody incubation concentration/times should be optimized by the user.

Chapter 2:
What You'll Need

Chapter Overview

- Required Consumables and Reagents
- Required Equipment
- Compatible Microarray Scanners
- Recommended Consumables & Equipment

Required Consumables and Reagents

Product Name	Company	Part Number	Details
scWest Kits • 10X Suspension Buffer • 5X Wash Buffer • Antibody Diluent 2 • Lysis/Run Buffer • 8 scWest chips	ProteinSimple	• K500 • K600 • K700 • K800	• Small scWest kit for small cells • Standard scWest kit • Large scWest kit for large cells • scWest calibration kit for unknown cell diameter
Primary antibodies	Various		
Fluorescently-conjugated secondary antibodies	Various		
Trypsin or other dissociation agent	Various		
10-cm Petri dishes (2 per scWest chip)	Various		

Table 2-1: *Required consumables and reagents.*

How to choose scWest chips based on cell size

Each scWest kit contains enough reagents for 8 scWest chip runs. One sample can be run on each scWest chip.

The K600 kit chips are appropriate for most cell types including HeLa, 293T, CHOK1, MCF-7, and other epithelial lines. The K500 kit is suitable for small cells, such as lymphocytes and other immune cells, while the K700 kit is suitable for larger than average cells. If the cells are of unknown diameter, calibration chips in the K800 kit are recommended to establish an optimal well diameter for your sample.

Cell Type	Diameter (µm)	scWest Chip
Dendritic Cell	7	Small
Lymphocyte	7	
Neutrophil	8	
Monocyte	9	
HT29	11	Standard
Jurkat	12	
PC12	12	
HEK293	13	
U87	13	
COLO-205	13	
CHO	14	
HUVEC	15	
A431	16	
K562	17	
Hela	18	
HepG2/C3A	18	
NIH/3T3	18	
SF-21	18	
U2OS	20	Large
Aveolar Macrophage	21	
COS-7	25	

Table 2-2: *Choosing scWest chips based on cell sizes.*

Required Equipment

- Milo
- Antibody incubation chamber and sponges (included with Milo, reorder PN A200)
- Metal tweezers for scWest chip handling (included with Milo)
- Benchtop vortexer
- Benchtop shaker
- 500 mL beaker/bottle for preparing 1X Suspension Buffer
- 500 mL beaker/bottle for preparing 1X Wash Buffer
- Brightfield cell culture microscope capable of 10X magnification
- Microarray scanner (see list of compatible scanners on page 9)
- Computer for data analysis using Scout software (Windows 7, Windows 8, or Mac OSX with 16-bit RAM or higher)
- Scout Software (download at proteinsimple.com/scout/downloads/)

Compatible Microarray Scanners

Scanner Name	Laser Count	Company
SpotLight Turbo	Up to 6	ArrayIT
SureScan	2 (simultaneous)	Agilent
InnoScan 710	2	Innopsys
InnoScan 910	3	Innopsys
InnoScan 1100AL	3	Innopsys
GenePix 4000B	2	Molecular Devices
GenePix 4100A	2	Molecular Devices
GenePix 4300A	Up to 4	Molecular Devices
GenePix 4400A	Up to 4	Molecular Devices
GenePix Professional 4200A	Up to 4	Molecular Devices
ScanArray Express	Up to 5	PerkinElmer
ScanArray Express HT	Up to 5	PerkinElmer
ScanArray 5000	4	PerkinElmer
LS Reloaded	Up to 4	Tecan
PowerScanner	2	Tecan
ArrayIT SpotLight	2	Telechem
GE Typhoon	3	General Electric
G2565CA	2	Agilent
Scanscope FL	4	Aperio

Table 2-3: *Compatible microarray scanners.*

Recommended Consumables & Equipment

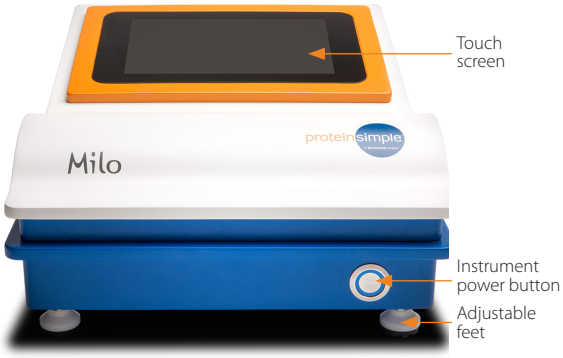
- Aluminum foil
- Microarray slide spinner (various suppliers)

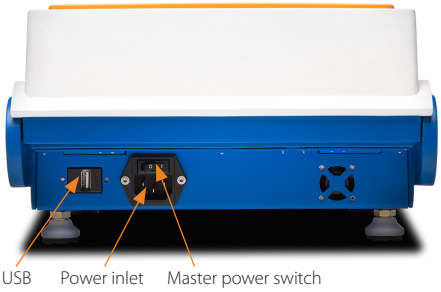
Chapter 3:
Milo

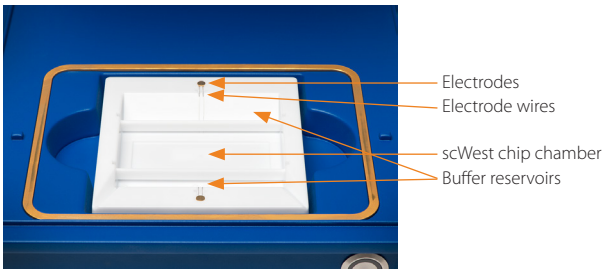
Chapter Overview

- Milo System Components
- Installation Requirements
- Physical Specifications
- Electrical Specifications
- Environmental Specifications
- Lamp Specifications

Milo System Components







Installation Requirements

Install Milo on a standard laboratory bench surface away from equipment that may create excessive vibration or shaking (e.g. large centrifuge). The bench surface must be flat and stable enough to accommodate leveling of Milo to +/- 0.5 degrees using the instrument's adjustable feet and internal electronic level.

Physical Specifications

Description	Specification
Depth	13.25 in (34 cm), allow ~18 in (~46 cm) total for ventilation and cable clearance at rear
Width	11 in (28 cm)
Height (lid closed)	6.5 in (17 cm)
Height (lid open)	12 in (31 cm)
Weight	<12 lb (<5.5 kg)

Table 3-4: *Milo dimensions and weight.*

Electrical Specifications

Description	Specification
Power	100-240 VAC input, 50/60 Hz 2A/1A Maximum rated input current
HV circuit rating	300 V, 2.5 A max

Table 3-5: *Milo voltage and current specifications.*

Environmental Specifications

Description	Specification
Operating temperature range	10–30 °C
Operating humidity range	10–90% RH (non condensing)
Pollution degree	2
Altitude	2000 m

Table 3-6: *Milo environmental specifications.*

Lamp Specifications

Description	Specification
UV Rating	240–400 nm, < 20 mW/cm^2

Table 3-7: *Milo lamp specifications.*

Chapter 4:

Experimental Setup

Chapter Overview

- How to Set Up Your Experiment
- Antibody Selection Guidelines

How to Set Up Your Experiment

One sample should be run per scWest chip. Triplicate chips can be run to increase the number of cells analyzed and ensure repeatability of quantitation results.

Antibody Selection Guidelines

Unlabeled primary antibodies (IgG isotype recommended) should be selected against each target of interest. For targets that have less than a 50 kDa molecular weight difference, primary antibodies must be from distinct host species (e.g., rabbit and mouse). For targets that have greater than a 50 kDa molecular weight difference, primary antibodies from the same host species (e.g., both rabbit) may be used. However, if two primary antibodies from the same host species are used, it is recommended to probe sequentially.

Secondary antibodies against each primary antibody host species should be chosen to have spectrally-distinct fluorescent dyes that match the laser and filter specifications of the microarray scanner that will be used to image the scWest chip.

Example antibody/dye configuration for a two-target experiment:

- **Primary antibodies:** Rabbit anti-Target 1 monoclonal antibody, mouse anti-Target 2 polyclonal antibody
- **Secondary antibodies:** Donkey anti-rabbit IgG conjugated to Cyanine 3, donkey anti-mouse IgG conjugated to Cyanine 5
- **Readout:** Using a two-laser scanner capable of Cyanine 3/Cyanine 5 imaging

Chapter 5:

Single-Cell Western Protocol

Chapter Overview

- Single-Cell Western Workflow
- Cell and Reagent Preparation
- Cell Loading and Quality Control
- Loading and Running Milo
- Antibody Probing
- Imaging scWest Chips

Single-Cell Western Workflow

The following step-by-step instructions summarize the basic steps needed to perform a Single-Cell Western with Milo (Figure 5-1).

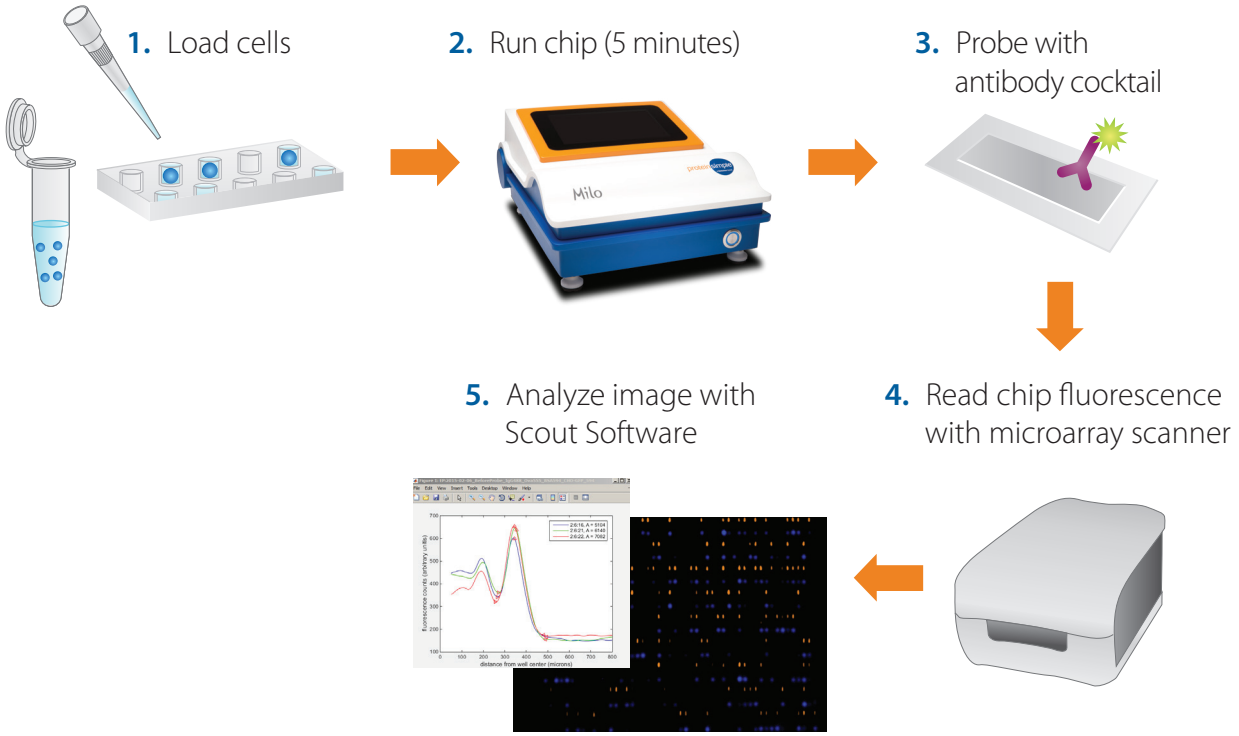


Figure 5-1: *Add 1 mL of a single-cell suspension onto an scWest chip. Individual cells settle into microwells patterned into the pre-cast polyacrylamide gel. Milo lyses the cells, does rapid (~1 min) SDS-PAGE on each single-cell lysate and immobilizes the proteins in the gel. Probe with conventional antibodies in the probing chamber and image chip fluorescence. Scout Software analyzes images to extract data.*

Cell and Reagent Preparation

A. Rehydrating scWest Chips

1 Determine how many scWest chips are needed for the experiment. One scWest chip is required for each sample to be analyzed.

NOTE: *scWest chips are currently available in four formats as listed in the Required consumables and reagents. table on page 6. If the cells are of unknown diameter, use the scWest calibration chip to determine the optimal microwell diameter. See the scWest Calibration Chip Quick Reference Guide for more information.*

2 Remove the correct number of scWest chips from the cannister and place each in a separate clean Petri dish with the gel facing up. The gel faces upwards when the "ProteinSimple" logo is legible. Take care not to touch the gel surface of the chip. If using nitrile gloves, wash gloved hands with soap and water before handling chips to minimize chip fouling.

3 Prepare 1X Suspension Buffer by adding 3 mL 10X Suspension Buffer to 27 mL DI water (30 mL total volume needed per chip).

4 Add 15 mL of 1X Suspension Buffer into each Petri dish to cover each scWest chip (Figure 5-2). Equilibrate all chips in 1X Suspension Buffer for >10 minutes (up to a maximum of 4 hours) at room temperature before use. This can be done prior to the single-cell suspension preparation, so that the chips are rehydrated by the time the single-cell suspension is prepared.

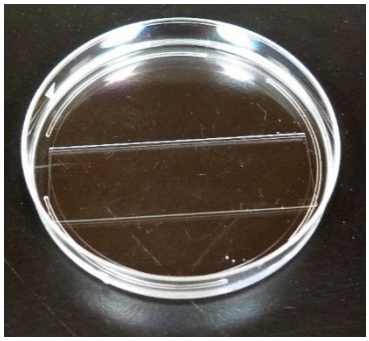


Figure 5-2: *scWest chip rehydrating in 15 mL of 1X Suspension Buffer.*

B. Preparing Wash Buffer

1 Prepare 1X Wash Buffer by adding 40 mL of 10X Wash Buffer to 160 mL DI water for a final volume of 200 mL. Excess 1X Wash Buffer can be stored at room temperature for future uses.

C. Preparing Cell Samples

1 Create a single-cell suspension using standard methods.

2 Centrifuge and wash the cell pellet with 5 mL 1X Suspension Buffer.

3 Centrifuge and re-suspend the cells in 1 mL 1X Suspension Buffer.

4 Count the cells using a hemocytometer and dilute to 100,000 cells/mL in 1X Suspension Buffer. Each scWest chip requires 1 mL of cell suspension containing 10,000–100,000 cells/mL.

Cell Loading and Quality Control

D. Loading Cells on the scWest Chip

1 Carefully aspirate the 1X Suspension Buffer from the Petri dish in which the scWest chip has been rehydrating.

NOTES:
Tilt the Petri dish so that the 1X Suspension Buffer pools to one side, then aspirate from the dish.

Remove excess droplets in the Petri dish without disturbing any 1X Suspension Buffer that remains beneath the scWest chip. If the chip is not flat when the cell suspension is added, the cells may not settle evenly across the chip. If air becomes trapped under the chip, additional 1X Suspension Buffer can be pipetted underneath the chip to re-balance it.

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2 Pipette 1 mL of the single-cell suspension drop-wise on top of the scWest chip in the Petri dish. The cell suspension should pool on top of the scWest chip and be sufficient volume to cover the gel surface without spilling over the chip (Figure 5-3).

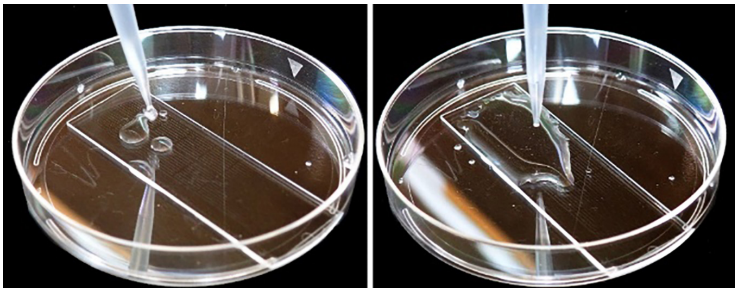

Figure 5-3: *Cells in 1X Suspension Buffer are steadily added (left) and form a pool on top of the scWest chip (~50% of cell suspension shown added to chip on the right). The cell suspension should entirely cover the chip after complete addition (not shown). If the cell suspension does not cover the entire chip, additional 1X Suspension Buffer can be added (<1 mL).*

3 Allow the cells to settle for 5–20 minutes at room temperature. Cell settling time should be optimized by the user and can be monitored by brightfield microscopy (Figure 5-4). Cells in microwells should appear out of focus with respect to cells that are floating or on the surface of the gel.

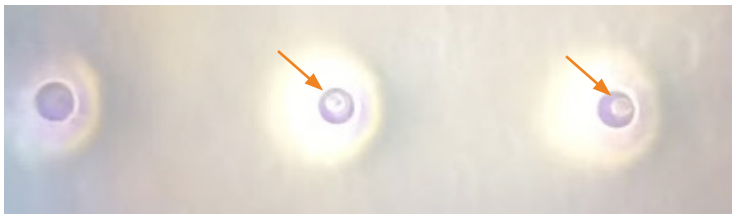

Figure 5-4: *Brightfield image of three wells. The well on the left is empty, while the central well and well on the right each contain one cell (arrows).*

NOTE: *Avoid excessive movement of the scWest chip as it can disturb cell settling. If the cell solution spills over the edge of the chip, continue incubation and check well occupancy after the desired settling time. If occupancy is low, the chip can be reloaded with an additional 1 mL of cell suspension. See "Milo Troubleshooting and FAQ" on page 39.*

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4 Gently tilt the scWest chip/Petri dish at a ~45° angle so the cell solution pools at the bottom of the chip (Figure 5-5).

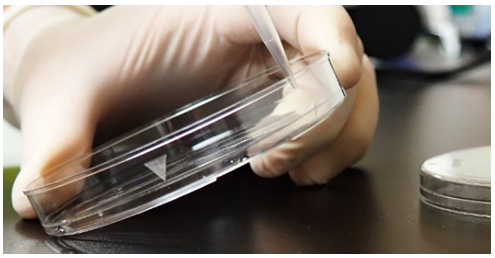

Figure 5-5: *Correct angle of scWest chip and Petri dish when collecting excess cells and performing washes.*

5 Aspirate the pooled cell solution from the Petri dish.

6 Still holding the Petri dish/scWest chip at ~45°angle, gently pipette 1 mL 1X Suspension Buffer on the top, short edge of the scWest chip to wash unsettled cells off of the gel surface.

7 Aspirate the pooled 1X Suspension Buffer from the wash step.

8 Repeat the washing step 1–2 more times as necessary to remove unsettled cells from the surface of the gel. Confirm that minimal cells remain on the gel surface using brightfield microscopy.

9 Estimate well occupancy by counting the number of wells in one block of a scWest chip that contain single cells and the number of wells that contain multiple cells. Count in a serpentine pattern through the block's 10 rows of 40 wells (Figure 5-6). Multiply the results from one block by 16 to get an estimate for the whole chip.

Optimal settling is achieved when a minimum of 10% of wells contain single cells and 2% or fewer wells contain multiple cells. Typical single-cell occupancies range from 10–25%. See "Milo Trouble-shooting and FAQ" on page 39.

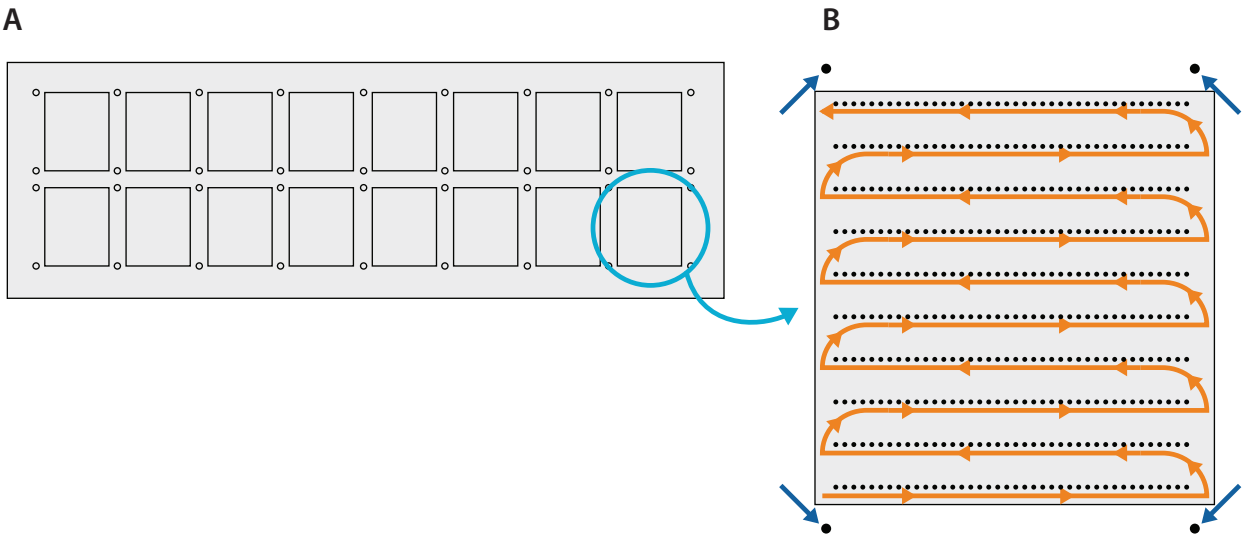

Figure 5-6: *Schematic of scWest chip architecture.* ***A:*** *The scWest chip is a 2x8 array of 400-well blocks separated by alignment markers (indicated by arrows). Each block contains 400 microwells arrayed in a 10-row by 40-well pattern.* ***B:*** *Typical layout of one block of an scWest chip (not to scale). Determine occupancy by first identifying an alignment marker (arrows) at the corner of a block, then counting the number of cells in each well of a block. This can be done rapidly by scanning across the block one row at a time in a serpentine fashion, as shown by the orange arrow.*

10 Proceed to the next step within 10 minutes to prevent the scWest chip from drying out. If the scWest chip does dry out, gently add 1–2 mL 1X Suspension Buffer to the surface of the scWest chip until the surface is covered, allow to sit for 1 minute, then gently tilt and aspirate the 1X Suspension Buffer before proceeding to the next step.

Proceed to the next step when optimal cell settling is achieved and minimal unsettled cells remain on the surface of the gel.

Loading and Running Milo

E. Inserting the scWest chip into Milo, setting run parameters and running Milo

!WARNING! SHOCK HAZARD
When operating, the pogo pin terminals may contain voltage up to 300 V at hazardous current levels. Do not directly touch the pogo pins. Never override the safety interlocks. The buffer solution is conductive. If spillage of buffer solution occurs outside of the sample area, immediately remove power to the unit and wait 30 seconds before opening the lid and cleaning the system.

CAUTION
Exposure to UV radiation can cause permanent damage to the eyes and skin. The UV transilluminator emits light in the range of 240–400 nm using a UV source which is not user serviceable/replaceable. This product is provided with dual redundant safety interlocks which prevent the UV source from operating when the lid is opened.

Never override the interlocks and operate the unit with the lid open.

1 Plug Milo into a power outlet using the provided power cord.

2 Turn on the master power switch at the back of the instrument.

3 Power on the instrument by pushing the power button on the front right of the instrument. Wait for the user interface to load on the touch screen (Figure 5-7).

Figure 5-7: *The power button for the instrument is on the bottom right side. Push to power on the instrument and touchscreen.*

4 Make sure the instrument is level by selecting the **Leveling** tab on the touchscreen interface. If the instrument is not completely leveled, adjust all four instrument feet until both electronic levels read a value of 0 ± 0.5 degrees. It is easiest to adjust the level one axis at a time left to right first, then back to front (Figure 5-8).

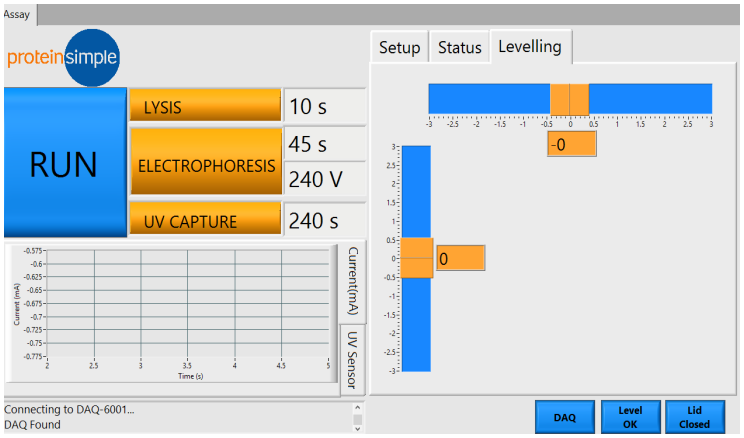

Figure 5-8: *Ensure the instrument is level and that the electronic levels both read 0 ± 0.5 degrees on the Leveling tab.*

5 Enter the desired lysis (Figure 5-9) and electrophoresis (Figure 5-10) parameters using Milo's touchscreen. Recommended initial values are given in Table 5-1. Enter the times in MM:SS format on the right side of the software interface. Values on the left side of the software interface will be used in the assay run.

IMPORTANT

DO NOT press the Run button at this step.

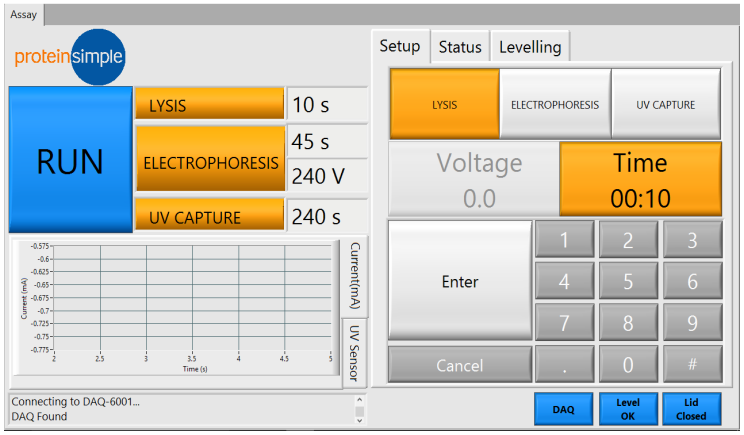

*Figure 5-9: To set the lysis time, press the **Lysis** button above the number pad, enter the desired length of time in seconds, then press **Enter**.*

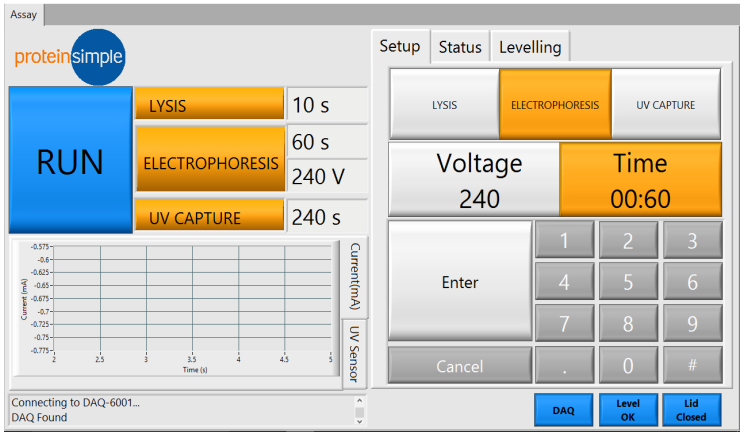

*Figure 5-10: To set the electrophoresis run parameters, press the **Electrophoresis** button above the number pad, enter the desired length of time (in minutes and/or seconds), and press **Enter**. Confirm that the Voltage is 240 V and that UV Capture is 240 sec.*

Target MW	Electrophoresis Time
10–30 kDa	45 seconds
30–80 kDa	60 seconds
80–175 kDa	90 seconds

Table 5-1: How to determine electrophoresis time. For multiple targets, choose the recommended electrophoresis time for the smallest target.

Recommended run parameters:

- **Lysis Time:** 10–15 seconds. Start with 10 seconds and adjust as needed.
- **Electrophoresis Time:** 45–90 seconds. Table 5-1 provides recommended electrophoresis times for varying target molecular weights.
- **Electrophoresis Voltage:** 240 V.
- **UV Capture:** 4 minutes (240 seconds).

See "Milo Troubleshooting and FAQ" on page 39 for more information on modifying Milo run parameters.

6 Open Milo's lid and verify that the electrophoresis cell is well-seated in the recessed area (Figure 5-11).

Figure 5-11: *Proper seating of the scWest chip and electrophoresis cell inside Milo.*

7 Pipette 300 µL of Lysis/Run Buffer on one end of the recessed region of the electrophoresis cell. Avoid/remove air bubbles. Carefully place the scWest chip in the electrophoresis cell with the gel side up and the bar code legible, starting with the end touching the drop of Lysis/Run Buffer and slowly lowering the scWest chip using the flat edge of the supplied tweezers until it is lying flat on a layer of buffer. The Lysis/Run Buffer should wick across the bottom of the chip. Avoid large air bubbles under the scWest chip.

NOTE: *At this step, avoid letting the Lysis/Run Buffer contact the gel layer on the top side of the chip.*

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8 Verify that Milo is ready by confirming the Level OK and DAQ icons on the lower part of the touchscreen are illuminated and that all run settings are correct.

9 IMPORTANT: Time-sensitive step! Pour the remaining aliquot of Lysis/Run Buffer into one of the two buffer reservoirs on the electrophoresis cell (Figure 5-12). Quickly verify that the entire scWest chip is covered by Lysis/Run Buffer.

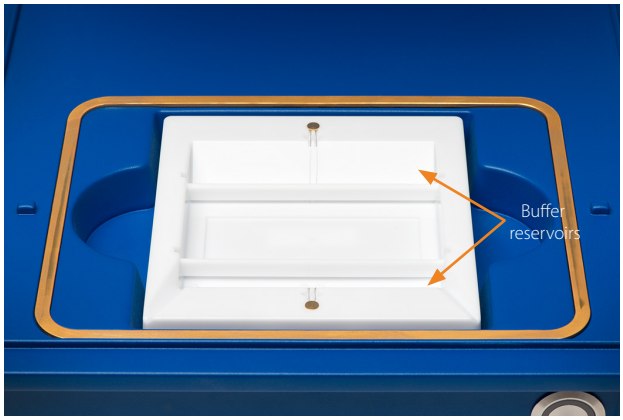

Figure 5-12: *Lysis/Run Buffer being added to the upper reservoir of the electrophoresis cell and spreading to cover a scWest chip. Reservoirs are indicated by arrows.*

10 Immediately close Milo's lid and press the **Run** button on the touch screen to initiate the run (Figure 5-13).

IMPORTANT

Lysis will begin immediately after buffer addition so it is important to initiate the run immediately after the Lysis/Run Buffer is added. Delays in starting the run may result in loss of cell lysate and poor run quality.

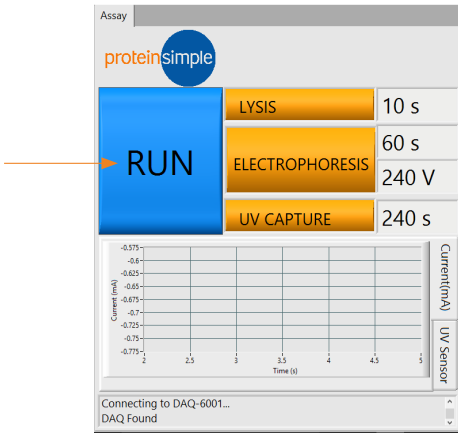

Figure 5-13: *Location of the Run button on Milo.*

11 Monitor the run progress by observing the count-down timers, UV flux and current traces on the left side of the screen. A typical run should take 5–6 minutes (Figure 5-14).

The current during electrophoresis will increase over the course of the run, but should stay in the range of 50–130 mA. If the current is significantly outside this range, check if the correct buffer was used.

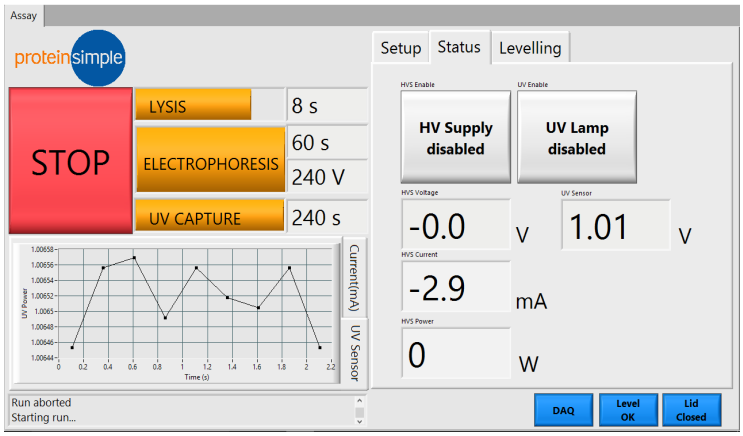

Figure 5-14: *The software interface during a run.*

12 Upon completion of the run, remove the scWest chip from Milo by gently lifting under one edge of the chip with the flat edge of the supplied tweezers. Do not attempt to pinch the chip with the tweezers as this will damage the gel layer. Place the chip in a new 10-cm Petri dish, gel-side up. Always use pristine new Petri dishes to avoid introducing dust or debris to the gel surface.

13 After each run, remove the electrophoresis cell from Milo and collect the Lysis/Run Buffer from the electrophoresis cell. Dispose of the Lysis/Run Buffer according to standard biohazardous waste disposal procedures. Use a DI water wash bottle to rinse the electrophoresis cell and remove any residual buffer and cell debris and then return it to Milo so it is ready for the next chip run. All free liquid should be removed from the electrophoresis cell before the next run, though it need not be completely dry to run the next scWest chip.

14 After all scWest chip runs are completed, the electrophoresis cell should be emptied, rinsed with DI water and allowed to dry completely before returning to Milo for storage.

Antibody Probing

F. scWest Chip Washing

1 Pour 15 mL of 1X Wash Buffer into the Petri dish to cover the scWest chip. Swirl to briefly wash the chip. Collect the 1X Wash Buffer for disposal according to standard biohazardous waste disposal procedures (e.g., bleach and drain disposal). Replace with 15 mL of fresh 1X Wash Buffer and incubate for 10 minutes on a shaker. Repeat for a total of two 10 minute washes.

NOTE: *Following the washes, unprobed chips can be stored in 1X Wash Buffer at 4 °C for 2–3 days or immediately probed for targets of interest. If storing chips for longer than 3 days before probing, dry the chips using the drying protocol on page 35 and rehydrate in 1X Wash Buffer for >10 minutes before probing.*

If probing immediately, proceed to the next steps.

G. Primary Antibody Probing

1 While the scWest chip is being washed, prepare the primary antibody cocktail solution. A total of 80 µL of prepared antibody solution is needed to probe each scWest chip. Dilute the primary antibodies to the desired final primary antibody concentration using Antibody Diluent 2 (see Table 5-2). Individual antibody concentrations must be determined by the user, but a suggested starting concentration is 100 µg/mL.

The composition of an example primary antibody cocktail solution in which two primary antibodies are used is shown below:

Component	1 mg/mL Ab 1	1 mg/mL Ab 2	Antibody Diluent 2	Total
Dilution	1:20	1:20	NA	NA
Volume	4 µL	4 µL	72 µL	80 µL

Table 5-2: *Composition of an example primary antibody cocktail solution.*

2 Place a probing chamber in a clean 15 cm Petri dish. Keep the probing chamber pristine to ensure uniform probing.

3 After scWest chip washing is complete, dab the edge of the chip on a laboratory wipe to remove excess Wash Buffer. The chip should be moist but without free liquid on the surface which will dilute the antibody solution during probing. Be careful not to touch the gel surface to the laboratory wipe as it can damage

or contaminate the gel. Pipette 80 µL of primary antibody solution into the primary antibody incubation chamber (Figure 5-15). Avoid/remove air bubbles as those will prevent antibody access to the gel.

4 Starting from one end, slowly lower the scWest chip onto the antibody incubation chamber with the gel facing downward (barcode not legible) as if you were applying a cover slip. Be careful not to introduce bubbles or apply shear stress to the gel (Figure 5-15).

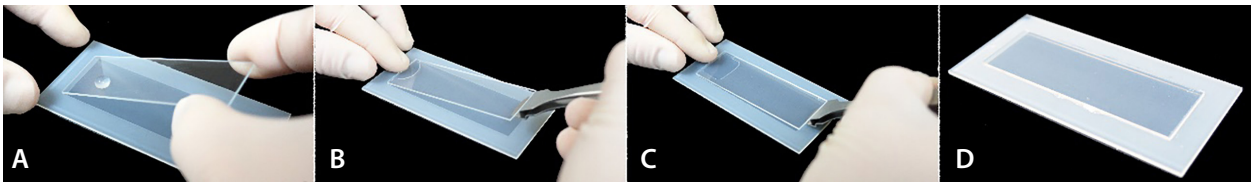

Figure 5-15: *Place the scWest chip in the incubation chamber.* ***A:*** *Pipette antibody cocktail solution in a bead on the left side of the incubation chamber.* ***B:*** *Gently lower the chip onto the antibody cocktail.* ***C:*** *Stabilize the scWest chip gel side down using the flat edge of tweezers (without gripping with the tweezers).* ***D:*** *scWest chip incubating in antibody cocktail solution with no air bubbles.*

5 While probing, cover the incubation chamber with an overturned Petri dish. Dampen the sponges provided and place them under the Petri dish to maintain humidity and prevent evaporation of the antibody solution.

6 Incubate the chip for 1–2 hours at room temperature. Antibody incubation times should be optimized by the user.

H. Washing

1 Remove the scWest chip from the primary incubation chamber. Gently push down on the top left or right corner of the chip with your middle finger. Gently lever tweezers under the diagonally-opposite corner of the scWest chip, using care not to scratch the underlying probing fixture (repeated damage to the probing fixture can result in decreased probing efficiency). Lift the chip up and remove it by pinching the opposite edges of the chip between your thumb and index finger. Place the chip in a clean Petri dish with the gel facing up.

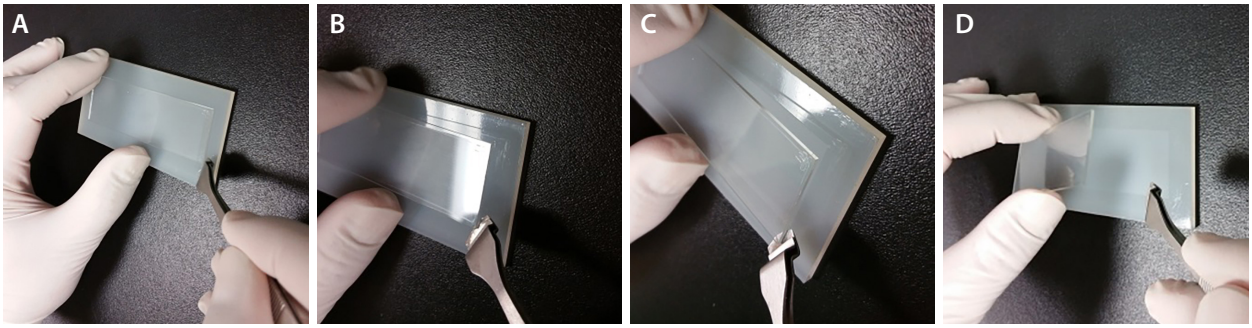

Figure 5-16: *Removing the scWest chip from the primary incubation chamber.* ***A:*** *Gently push down on the top corner of the chip with your middle finger.* ***B:*** *Lever the tweezers under the opposite corner of the chip without damaging the probing fixture.* ***C, D:*** *Remove the chip by lifting with the tweezers and pinching the opposite edges of the chip between your index finger and thumb.*

2 Pour 15 mL of 1X Wash Buffer into the Petri dish to cover the scWest chip. Place on a shaker and wash for 15 minutes.

3 Collect the 1X Wash Buffer for disposal according to standard biohazardous waste disposal procedures (e.g., bleach and drain disposal).

4 Repeat the wash step two additional times for a total of three15 minute washes.

I. Fluorescent Secondary Antibody Probing

1 While the scWest chip is in the final wash, prepare the secondary antibody solution. A total of 80 µL of prepared secondary antibody solution is needed per scWest chip. Dilute the secondary antibodies to the desired final secondary antibody concentration using Antibody Diluent 2 (see Table 5-3). Individual antibody concentrations must be determined by the user, but a suggested starting concentration for most secondary antibodies is 50 µg/mL.

Component	1 mg/mL Ab 1	1 mg/mL Ab 2	Antibody Diluent 2	Total
Dilution	1:40	1:40	NA	NA
Volume	2 µL	2 µL	76 µL	80 µL

Table 5-3: *Composition of an example secondary antibody solution in which two secondary antibodies are used:*

2 Turn the probing chamber over to use the other side for secondary antibody probing.

3 After the wash step is complete, dab the scWest chip on a laboratory wipe to remove excess Wash Buffer. The chip should be moist but without free liquid on the surface which will dilute the antibody solution

during probing. Be careful not to touch the gel surface to the laboratory wipe as doing so can damage or foul the gel. Pipette 80 µL of secondary antibody solution in a bead on one end of the secondary antibody incubation chamber (Figure 5-17). Remove any air bubbles, they will prevent antibody access to the gel.

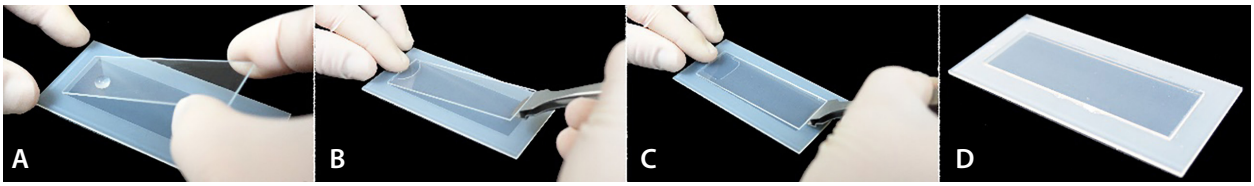

Figure 5-17: *Place the scWest chip in the incubation chamber.* ***A:*** *Pipette antibody cocktail solution in a bead on the left side of the incubation chamber.* ***B:*** *Gently lower the chip onto the antibody cocktail.* ***C:*** *Stabilize the scWest chip gel side down using the flat edge of tweezers (without gripping with the tweezers).* ***D:*** *scWest chip incubating in antibody cocktail solution with no air bubbles.*

4 Starting from one end, slowly lower the scWest chip onto the antibody incubation chamber with the gel facing downward as if you were applying a cover slip. Be careful not to introduce bubbles or apply shear stress to the gel (Figure 5-17).

5 Cover the incubation chamber with an overturned Petri dish. Dampen the sponges provided and place them under the Petri dish to maintain humidity and prevent evaporation of the antibody solution.

6 Incubate the chip for 1 hour at room temperature, protected from light. Antibody incubation times should be optimized by the user.

J. Washing

1 Remove the scWest chip from the secondary incubation chamber using tweezers and place in a clean Petri dish, gel side up (Figure 5-18).

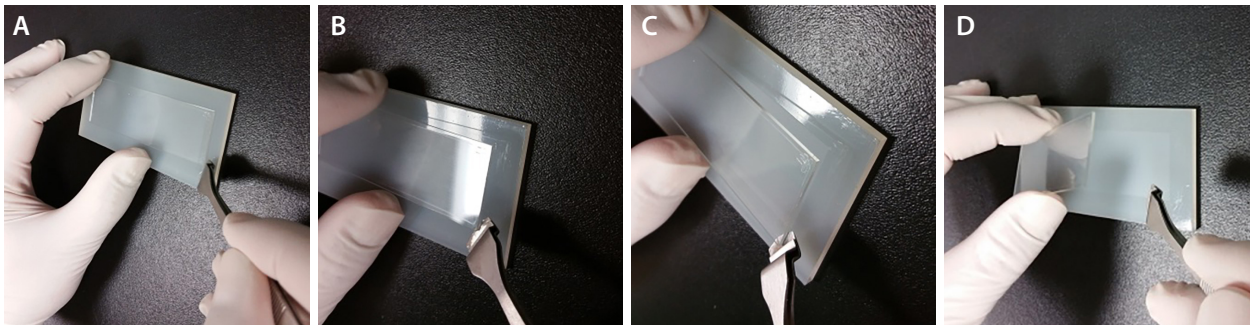

Figure 5-18: *Removing the scWest chip from the secondary incubation chamber.* ***A:*** *Gently push down on the top corner of the chip with your middle finger.* ***B:*** *Lever the tweezers under the opposite corner of the chip without damaging the probing fixture.* ***C, D:*** *Remove the chip by lifting with the tweezers and pinching the opposite edges of the chip between your index finger and thumb.*

2 Pour 15 mL of 1X Wash Buffer into the Petri dish to cover the scWest chip. Place on a shaker and wash for 15 minutes in the dark.

3 Collect the 1X Wash Buffer for disposal according to standard biohazardous waste disposal procedures (e.g., bleach and drain disposal).

4 Repeat the wash step two additional times for a total of three 15 minute washes.

Probed chips can be stored in 1X Wash Buffer at 4 °C for 2–3 days or dried and immediately imaged in a microarray scanner for targets of interest. If storing chips for longer than 3 days before scanning, dry the chips using the drying protocol on page 35.

NOTE: *For best results, scan scWest chips as soon as possible after probing (e.g., within 12–24 hours).*

Imaging scWest Chips

K. Scanning scWest Chips on a Microarray Scanner

1 Remove the scWest chip from the Petri dish and gently dab the glass edge of the chip dry using a laboratory wipe. Be careful not to touch the gel surface.

2 Rinse the chip in DI water to remove salts from the wash buffer. Recommended: repeat DI water wash two additional times for a total of three washes.

3 Recommended: spin the scWest chip with the gel side facing downward/inward for 3 min on an orbital slide spinner.

4 Recommended: air-dry the scWest chip by gently blowing compressed nitrogen or filtered compressed air at the surface of the chip for 1 minute. Make sure to dry the chip evenly and be careful not to damage it by drying too forcefully. Do not use unfiltered air straight from a compressor as particulates will contaminate the gel surface and result in unwanted noise upon scanning.

NOTE: *If compressed air is not available, spin the scWest chip in the orbital slide spinner for 8–10 minutes before scanning. If no orbital slide spinner is available, dry the chip for 3 minutes with filtered air. If neither a slide spinner nor compressed air is available, air dry the slide overnight protected from light and dust in a dry, uncovered Petri dish.*

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5 Turn on the microarray scanner and allow it to warm up for the recommended time (typically 5–30 minutes). Please consult the manual for your microarray scanner for exact times.

6 Place the scWest chip in the scanner face up or face down as appropriate to the type of scanner. Scan at 5 µm resolution at the appropriate wavelengths for the secondary antibodies used. For a list of compatible microarray scanners, please see Table 2-3, "Compatible Microarray Scanners" on page 9 .

7 Images for each spectral channel should be saved as single color TIFFs for analysis using Scout Software.

8 Imaged chips can be dried using the drying protocol and archived at room temperature, protected from light, for up to 9 months (see "scWest Chip Drying and Long Term Archiving Protocol" on page 35).

Chapter 6:

scWest Chip Storage and Archiving

Chapter Overview

- scWest Kit Storage
- scWest Chip Drying and Long Term Archiving Protocol

scWest Kit Storage

Upon receipt of a scWest kit, store the Antibody Diluent 2 at 2–8°C protected from light. Store the remaining kit components (scWest chips and buffers) at room temperature protected from light.

scWest Chip Drying and Long Term Archiving Protocol

1 In a Petri dish, rinse the chip in 15 mL of DI water for 10 minutes.

2 Spin the scWest chips dry for 3 min on a slide spinner. If no slide spinner is available, dry the underside of the scWest chip with a laboratory wipe after dabbing away any excess liquid.

3 Place the scWest chip gel side up in a clean, dry Petri dish without the lid and dry in the dark protected from dust at room temperature overnight. Alternatively, air-dry the scWest chip by gently blowing compressed nitrogen or filtered compressed air at the surface of the chip for 1 minute. Make sure to dry the chip evenly and be careful not to damage it by drying too forcefully. Do not use unfiltered air straight from a compressor as particulates will contaminate the gel surface and result in unwanted noise upon scanning.

4 Place the scWest chips in the provided chip holder and store in the dark at room temperature for up to 9 months. Re-hydrate chips in 1X Wash Buffer for >10 minutes before probing. Scanning chips after dry storage should be done without rehydrating the chip(s).

Chapter 7:

Image Analysis Using Scout Software

Chapter Overview

- Using Scout Software

Using Scout Software

1 Launch Scout Software

2 Under the File menu, add all scan images for a single chip by opening the saved TIFF files for each spectral channel.

3 Register each scan image to correct for image offsets or rotation by selecting two blocks for registration and clicking on the first well in the first registration block and last well in the last registration block.

4 The software will automatically identify all the lanes in an image and all the peaks in each lane using default settings. To adjust the detection settings, select **Edit > Scan Properties**. Once the peaks are detected properly, continue to the next step.

5 Visually inspect the detected peaks in each image to reject any false-positives owing to particulates or dust.

6 View the Peak Table to see all detected peaks in a spectral channel and tag the correct peaks as your target of interest. Peaks that lie outside the expected molecular weight range can be excluded.

7 Once all the appropriate peaks are detected, export the Peak Table to .csv or .fcs file formats to visualize your data in your favorite statistical software package.

For more information, please refer to the Scout Software User Guide.

Chapter 8:

Single-Cell Western Troubleshooting and FAQ

Chapter Overview

- Milo Troubleshooting and FAQ

Milo Troubleshooting and FAQ

Question/Problem	Possible Cause/Issue	Solution
How do I determine which scWest chip to use?	Cell size unknown.	The scWest calibration chip can be used to determine which scWest chip size to use.
How do I use the scWest calibration chip?	First scWest chip run.	Refer to the scWest Calibration Chip Quick Reference Guide for more information.
Can I load fewer than 100,000 cells onto each scWest chip?	Low number of cells available.	Lower starting cell numbers can be used either by loading down to 10,000 cells in 1 mL or by decreasing the volume of cell suspension and settling over a smaller area on a dry chip. Optimal cell settling should be confirmed using brightfield microscopy.
My cell suspension did not spread across the entire chip.	Poor chip rehydration.	Add additional (<1 mL) 1X Suspension Buffer to cover the surface of the chip. ***NOTE:*** scWest chips should be rehydrated in 1X Suspension Buffer for at least 10 min prior to cell settling.
	Chip was gel face-down.	Confirm that chip is gel face-up before adding cells.
How should cell settling time be determined?	Too many or too few cells in microwells.	Optimal cell settling is achieved when a minimum of 10% of wells contain single cells and 2% or fewer wells contain multiple cells. Typical single-cell occupancies range from 10–25%. Increasing settling time will lead to increased occupancy, but may increase the number of wells with more than one cell.

Milo Troubleshooting and FAQ, *continued*

Question/Problem	Possible Cause/Issue	Solution
What if less than 10% of my wells contain a single cell?	Too few cells added.	Increase number of cells added.
	Cell settling time was too short.	Increase cell settling time.
	Cells were too large for chip well size.	Select a scWest chip with larger wells.
	Cells were not in single-cell suspension (e.g., clumped cells).	Triturate cells to create single-cell suspension.
	Chip was gel face down.	Confirm that chip is gel face-up before adding cells.
What if more than 2% of my wells contain two or more cells?	Too many cells added.	Decrease number of cells added.
	Cell settling time was too long.	Decrease cell settling time.
	Cells were too small for chip well size.	Select an scWest chip with smaller wells.
Can phosphorylated proteins be detected using Milo?	N/A	Yes, Milo enables detection of phosphorylated proteins. To preserve phosphorylation states, add 1 mM sodium fluoride and 1 mM sodium orthovanadate to the Lysis/Run Buffer just prior to addition.
Protein signal is only near well.	Incomplete lysis.	Increase lysis time.
	Electrophoresis too short.	Increase electrophoresis run time.
Protein signal is smeared across the lane starting from well.	Incomplete lysis. Continuous protein injection.	Lysis time should be increased.
	Antibody binding off target to background cell lysate.	Antibody concentrations might be too high and should be decreased.
Protein signal too close to next well or into next lane.	Electrophoresis time too long.	Decrease electrophoresis time.
Native fluorescent signal weak/not appearing.	Electrophoresis time too long.	Decrease electrophoresis time.
	Photobleaching due to UV exposure.	Decrease UV exposure time to 1 minute.

Milo Troubleshooting and FAQ, *continued*

Question/Problem	Possible Cause/Issue	Solution
Air bubbles while probing with antibodies.	Air bubbles in antibody droplets.	Avoid air bubbles in the antibody droplets when preparing the antibody solution.
	scWest chip laid down poorly in antibody probing fixture.	scWest chips must be lowered slowly in the antibody probing fixture to allow antibody spreading without air bubble formation.
No signal for protein target.	Antibody concentration too low.	Increase primary and/or secondary antibody concentration.
	Poor antibody.	Try a different Western-validated antibody.
	Probing orientation.	Confirm gel is face-down during antibody probing.

Chapter 9:

General Information

Chapter Overview

- Compliance
- Safety
- Safety Data Sheets
- Instrument Cleaning and Decontamination
- Customer Service and Technical Support
- Legal Notices

Compliance

__NOTE:__ This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his/her own expense.

- This Class A digital apparatus complies with Canadian ICES-003. Cet appareil numérique de la classe A est conforme à la norme'.
- **EN 61010-1:2010 (Third Edition):** Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements (EU)
- **EN 61326-1:2013:** Electrical equipment for measurement, control and laboratory use. EMC Requirements. General requirements (EU)

CE

Safety

As with all experiments, please adhere to general lab safety guidelines including, but not limited to, the following:

- Wear personal protective equipment (PPE) including safety glasses, fully enclosed shoes, long pants, and gloves.
- Know the locations of all safety equipment including fire extinguishers, spill kits, eyewashes/showers, first aid kits, and emergency/injury reporting procedures.

Explanation of Symbols Used

The following safety alert labels are located on Milo or are displayed in the user guide indicating a potential safety hazard.

Symbol	Description
	**CAUTION** Performing or omitting a specific action may result in equipment damage or injury.
	**CAUTION** Performing or omitting a specific action may result in exposure to hazardous ultraviolet (UV) radiation.
	**!WARNING!** Performing or omitting a specific action may result in electrical shock.

Safety Guidelines

!WARNING!
To prevent electric shock, do not remove the covers. No user serviceable parts inside. The unit contains hazardous voltages/currents and should only be opened by a trained service person. To avoid the possibility of electric shock, remove the power cord before servicing.

!WARNING! SHOCK HAZARD
When operating, the pogo pin terminals may contain voltage up to 300 V at hazardous current levels. Do not directly touch the pogo pins. Never override the safety interlocks. The buffer solution is conductive. If spillage of buffer solution occurs outside of the sample area, immediately remove power to the unit and wait 30 seconds before opening the lid and cleaning the system.

!WARNING! CLASS 1 EQUIPMENT
This equipment must be earthed. The power plug must be connected to a properly wired earth grounded socket outlet.

Pluggable Equipment: The socket outlet should be installed near the equipment and be easily accessible.

!WARNING!
If Milo is not used as specified by ProteinSimple, overall safety will be impaired.

!WARNING!
If Milo is damaged and doesn't function properly, stop him safely and contact ProteinSimple Technical Support right away.

CAUTION
Protection may be impaired if this device is used in a manner not specified by the manufacturer.

CAUTION
Exposure to UV radiation can cause permanent damage to the eyes and skin. The UV transilluminator emits light in the range of 240–400 nm using a UV source which is not user serviceable/replaceable. This product is provided with dual redundant safety interlocks which prevent the UV source from operating when the lid is opened.

Never override the interlocks and operate the unit with the lid open.

Chemical Hazards

!WARNING! CHEMICAL HAZARD
Some chemicals used can be potentially hazardous, and can cause injury or illness.

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or clothing). For additional safety guidelines, consult the SDS.
- Do not leave chemical containers open.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup Procedures as recommended on the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Safety Data Sheets

Some chemicals used with Milo may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

SDSs provide users with safety information needed to store, handle, transport and dispose of the chemicals safely. We recommend updating laboratory SDS records periodically.

Safety Data Sheets for ProteinSimple reagents are available online at www.proteinsimple.com/literature or by calling (888) 607-9692. Otherwise, call the chemical manufacturer directly or visit their website.

Instrument Cleaning and Decontamination

Milo can be cleaned by wiping hard surfaces with a soft cloth saturated in 70% ethanol.

NOTE: *Do not spray liquids directly onto the instrument.*

Customer Service and Technical Support

Telephone
(408) 510-5500
(888) 607-9692 (toll free)

Fax
(408) 510-5599

E-mail
support@proteinsimple.com

Web
www.proteinsimple.com

Address
ProteinSimple
3001 Orchard Pkwy
San Jose, CA 95134
USA

Legal Notices

NOTE: *Read the Legal Notices carefully before using Milo and Scout software.*

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2.4 Ownership. Company reserves all rights not expressly granted to Customer in this Agreement. Without limiting the generality of the foregoing, Customer acknowledges and agrees that, except as expressly set forth in this Agreement, Company and its suppliers retain all Intellectual Property Rights, title and interest in and to the Licensed Software and Documentation.

3. Support and Maintenance Services

3.1 Services. Subject to Customer's payment of the Services fees, as set forth in the System Quotation, and to the terms and conditions herein, Company will use commercially reasonable efforts to provide to Customer the following support and maintenance services (the "Services") for the Licensed Software: (a) Company will answer technical questions concerning functions and features of the Licensed Software; (b) Company will provide Error verification, analysis and corrective efforts for the Licensed Software; and (c) Company will provide, without charge, Updates of the software released during the term of this Agreement. Customer will be responsible for providing, in a manner consistent with good industry practice, all Services to Users. Customer acknowledges that Company may not be able to correct all reported Errors. Any Update of the Licensed Software will be deemed part of the Licensed Software and Customer will use such Updates in accordance with the requirements and obligations in this Agreement.

3.2 Service Conditions. Company's obligation to provide the Services is conditioned on Customer: (a) notifying Company of any Error within a reasonable period of time; (b) providing Company all information relating to the Error; (c) providing access to the Licensed Software and Customer's facility where the Licensed Software is located and informing Company of any potential hazards which may be encountered while servicing the Licensed Software. Customer may contact Company via telephone at

1-888-607-9692 or e-mail at support@proteinsimple.com during the hours of 8 a.m. (Pacific Time) and 5 p.m. (Pacific Time) Monday through Friday, excluding holidays, to report any Error. A list of standard holidays will be provided to Customer upon request. Company shall have the right to determine in its sole discretion what corrective action Company will perform to support the Licensed Software. Company may subcontract the Services to a third party contractor provided that Company will be responsible for the third party contractor's compliance with this Agreement.

3.3 Service Exclusions. Company will not be obligated to provide the Services if (a) Company determines that an Error is caused by malfunction of any hardware (other than malfunction of the System) or third party software used with the Licensed Software; or (b) Customer has failed to incorporate the latest Update previously released to Customer.

4. Warranty

4.1 Licensed Software Warranty. Company warrants that the Licensed Software, as properly installed, and under normal use, will perform substantially in accordance with its Documentation during the Warranty Period. The "Warranty Period" for the Licensed Software begins on date Customer downloads the Licensed Software and ends twelve (12) months thereafter.

4.2 Remedy. If Customer notifies Company in writing during the Warranty Period of an Error, Company will, at its expense and as its sole obligation for any breach of the foregoing warranty, use commercially reasonable efforts to correct the Error or replace the Licensed Software. Any Error correction or replacement of the Licensed Software will not extend the original Warranty Period. The warranty and the remedies provided above will not apply to the Licensed Software if (a) Company determines that an Error is caused by accident, abuse, misuse, negligence, fire, earthquake, flood, other force majeure event, failure of electrical power, the use of unauthorized products, or unauthorized repairs or modifications; (b) Company determines that an Error is caused during or as a result of delivery; (c) a problem arises from or is based on Company's compliance with Customer's specifications; or (d) Company determines that an Error is caused by malfunction of any hardware (other than malfunction of the System) or third party software used with the Licensed Software.

4.3 Disclaimer. THE WARRANTIES ABOVE ARE EXCLUSIVE AND IN LIEU OF ALL OTHER WARRANTIES, WHETHER EXPRESS, IMPLIED OR STATUTORY, INCLUDING WITHOUT LIMITATION THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, TITLE, AND NONINFRINGEMENT.

5. Limitation of Liability. NEITHER COMPANY NOR ITS SUPPLIERS SHALL BE RESPONSIBLE OR LIABLE WITH RESPECT TO ANY SUBJECT MATTER OF THIS AGREEMENT OR TERMS OR CONDITIONS RELATED THERETO UNDER ANY CONTRACT, NEGLIGENCE, STRICT LIABILITY OR OTHER THEORY (A) FOR LOSS OR INACCURACY OF DATA, LOSS OF PROFITS OR COST OF PROCUREMENT OF SUBSTITUTE GOODS, SERVICES OR TECHNOLOGY, OR (B) FOR ANY INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES INCLUDING, BUT NOT LIMITED TO LOSS OF REVENUES AND LOSS OF PROFITS. COMPANY'S AGGREGATE CUMULATIVE LIABILITY HEREUNDER SHALL NOT EXCEED THE GREATER OF FIVE HUNDRED DOLLARS ($500.00).

6. Term and Termination

6.1 Term of Agreement. The Agreement is effective on the date Customer downloads the Licensed Software and shall remain in effect until terminated by either party as provided in this section.

6.2 Termination For Material Breach. Either party may terminate this Agreement upon written notice if the other party materially breaches this Agreement and fails to cure such breach within thirty (30) calendar days following receipt of written notice from the other party specifying the breach in detail. Notwithstanding the foregoing, Company may immediately terminate this Agreement and all licenses granted hereunder if Customer breaches Section 2 (License and Restrictions) hereof or upon termination of the System Quotation. The foregoing rights of termination are in addition to any other rights and remedies provided in this Agreement or by law.

6.3 Effect of Termination. Upon termination of this Agreement (or termination or expiration of any license granted hereunder), all rights of Customer to use the Licensed Software and Documentation will cease and (a) all license rights granted under this Agreement will immediately terminate and Customer shall promptly stop all use of the Licensed Software and Documentation; (b) all Services will terminate immediately; (c) Customer shall promptly erase all copies of the Licensed Software from Customer's computers, and destroy all copies of the Licensed Software and Documentation on tangible media in Customer's possession or control or return such copies to Company; and (d) upon request by Company, Customer shall certify in writing to Company that it has returned or destroyed such Licensed Software and Documentation. The parties' rights and obligations under Sections 1 (Definitions), 2.4 (Ownership), 4.3 (Disclaimer), 5 (Limitation of Liability), 6 (Term and Termination), and 7 (General) shall survive termination of this Agreement.

7. General

7.1 Assignment. This Agreement and Customer's rights hereunder may not be assigned to any third party by Customer except with the prior written approval of Company. Any attempted assignment of this Agreement or any rights or obligations hereunder will be null and void.

7.2 Governing Law. This Agreement is made in, governed by, and shall be construed in accordance with the laws of the State of California, without regard to any conflicts of law principles that would result in application of laws of any other jurisdiction. The United Nations Convention on Contracts for the International Sale of Goods does not apply to this contract. Any legal action or other legal proceeding relating to this contract or the enforcement of any provision of this contract must be brought in any state or federal court located in Santa Clara County, California. Customer and Company expressly and irrevocably consents and submits to the jurisdiction of such courts.

7.3 Injunctive Relief. Customer acknowledges that the Licensed Software contains valuable trade secrets and proprietary information of Company, that any actual or threatened breach of this Agreement will cause harm to Company for which monetary damages would be an inadequate remedy, and that injunctive relief is an appropriate remedy for such breach.

7.4 Modifications. Company reserves the right to change the terms and conditions of this Agreement or its policies relating to the Licensed Software at any time. Company will notify Customer of any material changes to this Agreement by sending Customer an e- mail to the last e-mail address Customer provided to Company or by prominently posting notice of the changes on Company's website. Any material changes to this Agreement will be effective upon the earlier of thirty (30) calendar days following Company's dispatch of an e-mail notice to Customer or thirty (30) calendar days following Company's post-

ing of notice of the changes on Company's website. These changes will be effective immediately for new users of our Licensed Software. Please note that at all times Customer is responsible for providing Company with its most current e-mail address. In the event that the last e-mail address that Customer has provided Company is not valid, or for any reason Company is not capable of delivering to Customer the notice described above, Company's dispatch of the e-mail containing such notice will nonetheless constitute effective notice of the changes described in the notice. If Customer does not agree with the changes to this Agreement, Customer must notify Company prior to the effective date of the changes that Customer wishes to terminate its license to the Licensed Software. Continued use of the Licensed Software, following notice of such changes, shall indicate Customer's acknowledgement of such changes and agreement to be bound by the terms and conditions of such changes.

7.5 Severability. In the event any provision of this Agreement is held to be invalid or unenforceable, the remaining provisions of this Agreement will remain in full force.

7.6 Waiver. The waiver by either party of any default or breach of this Agreement shall not constitute a waiver of any other or subsequent default or breach.

7.7 Export. Customer agrees not to export, reexport, or transfer, directly or indirectly, any U.S. technical data acquired from Company, or any products utilizing such data, in violation of the United States export laws or regulations.

7.8 Force Majeure. Company shall not be liable, directly or indirectly, for any delay or failure in performance of any obligation under this Agreement, including any delivery obligation, where such delay or failure arises or results from a cause beyond Company's reasonable control, or beyond the reasonable control of Company's suppliers or contractors, including, but not limited to strike, boycott or other labor disputes, embargo, governmental regulation, inability or delay in obtaining materials, acts of God, war, earthquake, fire, or flood. In the event of such force majeure, the time for delivery or other performance will be extended for a period equal to the duration of the delay caused thereby, provided that Company notifies Customer of the nature and duration of such force majeure event.

7.9 Entire Agreement; Notice. This Agreement constitutes the complete agreement between the parties and supersedes all prior or contemporaneous agreements or representations, written or oral, concerning the subject matter of this Agreement. Except as otherwise expressly provided in this Agreement, any modifications of this Agreement must be in writing and agreed to by both parties. Company may provide any notice to Customer by e-mail. Customer may provide notice to Company by sending an e-mail to info@proteinsimple.com or a letter by United States mail to ProteinSimple, 3040 Oakmead Village Drive, Santa Clara, CA 95051, or to such other address as Company may specify in writing by posting the new address on the Company website.

7.10 Relationship of the Parties. The parties are acting hereunder as independent contractors and not as partners, agents, fiduciaries, or joint venturers. Neither party has the power or authority represent, act for, bind, or otherwise create or assume any obligation on behalf of the other party.