Milo

Standard scWest Kit

Part# K600

Let's get started!

The Standard scWest Kit contains everything you need to run eight single-cell western assays on the Milo™ system using standard diameter cells.

Components

INCLUDES	PART NO.	QTY/KIT
scWest Chips — Standard	C300	8
10X Suspension Buffer	R101	50 mL
5X Wash Buffer	R252	250 mL
Lysis/Run Buffer (single use)	R200	8 x 15 mL
Antibody Diluent 2	042-203	1
Milk-Free Antibody Diluent	043-524	1

Storage conditions

- Store the Antibody Diluent 2 and Milk-Free Antibody Diluent at 2–8 °C. Everything else should be stored at room temperature.

A few things you should know

- When handling scWest chips, be careful not to touch the gel surface of the chip. Always use pristine, clean Petri dishes and take care not to introduce dust or particulates that can foul the gel surface.
- Before running the scWest chips, rehydrate them in 1X Suspension Buffer in a 10 cm Petri dish for >10 min at room temperature.
- If you are storing scWest chips for >1 day, store them dry and protected from light and dust. Please see the Milo User Guide for more details.
- If goat or sheep primary antibodies will be used in any probing cycle, dilute the primary and secondary antibodies in Milk-Free Antibody Diluent (P/N 043-524). If goat or sheep primary antibodies will not be used, dilute all antibodies in Antibody Diluent 2 (P/N 042-203).

Other things you'll need

- Milo instrument, PN P100
- Tweezers (supplied with Milo)
- Scout Software (download from ProteinSimple website)
- Probing chamber and sponges (supplied with Milo, PN A200)
- Trypsin or other dissociation agent
- Primary antibodies
- Fluorescently-labeled secondary antibodies
- 2 x 500 mL beaker/bottles for buffer reconstitution
- Pipettes and tips
- 16 x 10 cm Petri dishes
- Assorted Eppendorf tubes, 15 mL and 50 mL conical tubes
- Aluminum foil (optional)
- Brightfield cell culture microscope capable of 10X magnification
- Centrifuge
- Benchtop vortexer
- Benchtop shaker
- Microarray slide spinner (P/N 110-0006 for 120V option, P/N 110-0007 for 230V option)
- Compatible microarray scanner to image scWest chips
- Computer for image analysis

1. Prepare your reagents and samples

A PREPARE YOUR 1X SUSPENSION AND WASH BUFFERS

1. Prepare 1X Suspension Buffer with DI water
2. Prepare 1X Wash Buffer with DI water.

B REHYDRATE YOUR scWEST CHIPS IN 1X SUSPENSION BUFFER

1. Determine how many scWest chips are needed for your experiment.
2. Remove the correct number of scWest chips from the canister and lay them each in a separate, pristine 10 cm Petri dish with the gel facing up. If the logo is on the left and the barcode on the right, the gel is facing up (**Figure 1**). Be careful not to touch the gel surface of the chips or introduce any dust or debris. Wash gloved hands before handling scWest chips.

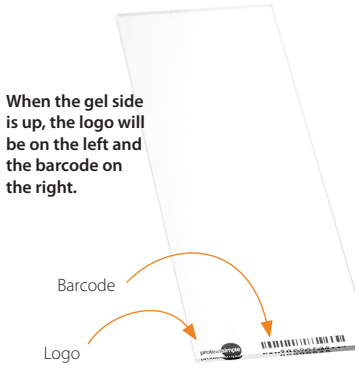

Figure 1. scWest gel orientation.

3. Add 15 mL of 1X Suspension Buffer to each Petri dish so that each scWest chip is completely covered.
4. Equilibrate all scWest chips in 1X Suspension buffer for >10 minutes before use.

C PREPARE YOUR SINGLE-CELL SUSPENSION

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 and dilute to 10K–100K/1 mL concentration with 1X Suspension Buffer. Prepare sufficient volume to load 1 mL of cell suspension per scWest chip. For example, if you are running 2 scWest chips, dilute cells to 2+ mL total volume at a concentration of 100,000 cells/mL.

2. Load scWest Chips and run them on Milo

D LOAD CELLS ON YOUR scWEST CHIP

1. Load 1 mL cell suspension dropwise on top of the scWest chip.
2. Let the cells settle for 5–20 minutes.
3. Wash off unsettled cells with 1X Suspension Buffer.
4. Score microwell occupancy using a brightfield microscope by counting occupancy for 1 block of 400 microwells. The goal is for 15–20% of microwells to contain 1 single cell and <2% to contain more than one single cell.

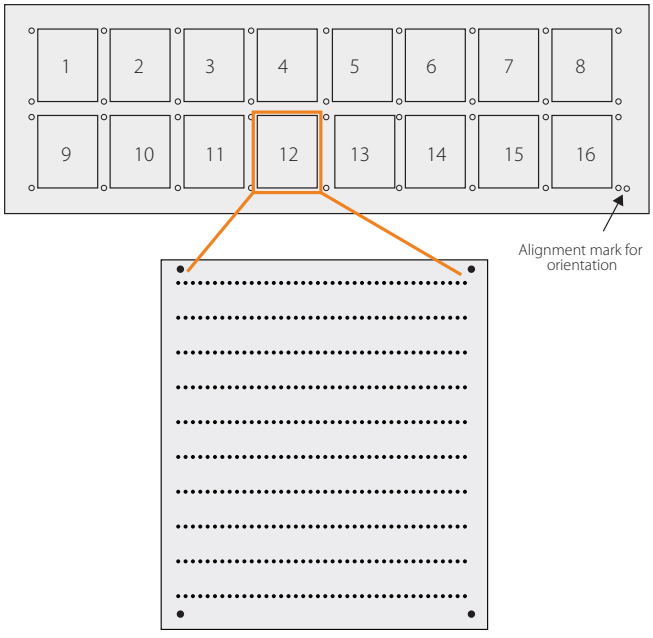

Figure 2. scWest chip layout with block numbering.

E RUN YOUR scWEST CHIPS ON MILO

1. Pipette 300 µL of Lysis/Run Buffer on one end of the recessed region of the electrophoresis cell. Avoid/remove bubbles.
2. Place a scWest chip gel side up in Milo and verify the user-optimized run settings. Recommended starting run settings:

TYPICAL MILO SETTINGS	VALUE
Lysis time	0–15 seconds
Electrophoresis run time	1 minute
Electrophoresis voltage	240 V
UV exposure time	4 minutes

3. Pour the remaining aliquot of lysis/run buffer into one of the reservoirs of the electrophoresis cell. Quickly close the lid and press the Run button.
4. Remove the scWest chip from Milo and wash in 1X Wash Buffer on a shaker for 2x10 minutes.
5. Repeat as needed with remaining scWest chips.

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 in the Milo User Guide and rehydrate in 1X Wash Buffer for >10 minutes before probing.

3. Probe scWest chips with primary and fluorescent secondary antibodies

F PROBE scWEST CHIPS WITH PRIMARY ANTIBODIES

1. Prepare 80 µL of primary antibody cocktail solution per scWest chip. Typical primary antibody dilutions range from 1:5 to 1:20. An example primary antibody cocktail is shown in the table below.
   - Milk-Free Antibody Diluent should be used in primary antibody cocktails if a goat or sheep primary antibody is used in any probing cycle. Otherwise, use Antibody Diluent 2.

COMPONENT	1 mg/mL AB 1	1 mg/mL AB 2	ANTIBODY DILUENT	TOTAL
Dilution	1:20	1:20	NA	NA
Volume	4 µL	4 µL	72 µL	**80 µL**

2. Remove the chip from the Wash Buffer and tap off excess buffer. Place the chip in a slide spinner and spin dry for 3-5 sec. Lay the chip gel side down onto a clean, dry antibody probing chamber. Inject up to 80 µL of primary antibody solution in the middle of the long edge of the chip into the gap between the chamber and the edge of the chip so that the antibody solution fills the chamber completely. Avoid introducing air bubbles or overfilling.
3. Incubate the scWest chips with the primary antibody at room temperature on the bench for 1–2 hours.
4. Wash 3 x10 minutes in 1X Wash Buffer on a shaker.

G PROBE scWEST CHIPS WITH SECONDARY ANTIBODIES

1. Prepare 80 µL of secondary antibody cocktail solution per scWest chip. Typical secondary antibody dilutions range from 1:10 to 1:40. An example secondary antibody cocktail is shown in the table below.
   - Use the same antibody diluent as you used for your primary antibody cocktail.

COMPONENT	1 mg/mL AB 1	1 mg/mL AB 2	ANTIBODY DILUENT	TOTAL
Dilution	1:40	1:40	NA	NA
Volume	2 µL	2 µL	76 µL	**80 µL**

2. Turn the probing chamber over. Remove the chip from the Wash Buffer and tap off excess buffer. Place the chip in a slide spinner and spin dry for 3-5 sec. Lay the chip gel side down onto a clean, dry antibody probing chamber. Inject up to 80 µL of secondary antibody solution in the middle of the long edge of the chip into the gap between the chamber and the edge of the chip so that the antibody solution fills the chamber completely. Avoid introducing air bubbles or overfilling.
3. Incubate the scWest chips with the secondary antibody at room temperature on the bench for 1 hour protected from light.
4. Wash 3x15 minutes in 1X Wash Buffer on a shaker protected from light.

4. Image scWest chips and analyze data with Scout Software

H SCAN scWEST CHIPS ON A FLUORESCENCE MICROARRAY SCANNER

1. Rinse scWest chip 3 times with DI water to remove Wash Buffer. Dry each scWest chip in a microarray slide spinner for 3 minutes.
2. Image the scWest chips on a compatible fluorescence microarray scanner with 5 µm scanning resolution. For a list of compatible scanners, please consult proteinsimple.com/milo.
3. For each color you scan on each scWest chip, save the image as a single-color 16-bit TIFF file.

I ANALYZE YOUR DATA WITH SCOUT SOFTWARE

1. Open the single-color TIFF files in Scout Software.
2. Align the images using Scout's prompts so that he can automatically detect all 6,400 lanes on each chip and detect all protein bands in each lane.
3. AutoTag noise peaks and label your protein bands of interest.
4. Visualize your data using the visualization tools in Scout or export the data to .csv or .fcs files so you can plot it in your favorite data visualization tool.

That's it, you're done!

Milo

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