Milo Three-Plex Antibody Probing Fixture
Quick Reference Guide

proteinsimple
a bio-techne brand

SPEED UP YOUR ANTIBODY VALIDATION!

Use a Three-Plex Antibody Probing Fixture (**Figure 1**) to shorten your assay development time. This probing fixture allows you to screen three antibody cocktails simultaneously in a single population of cells by dividing up the gel surface into three distinct regions. If a four-color scanner is available, this allows for screening up to 12 different antibodies on a single scWest chip.

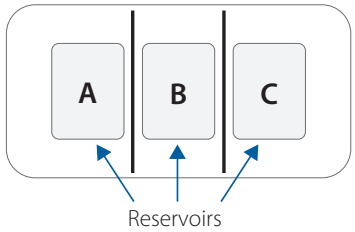

Figure 1. Three-Plex Probing Fixture, containing three reservoirs where different antibody solutions can be applied.

1. PREPARE YOUR scWEST CHIP

1.1 After running your scWest chip in Milo, wash it 2x10 min in Wash Buffer as normal.

1.2 Prepare three antibody cocktail solutions (one per reservoir) in Antibody Diluent 2, each with a total volume of 40 µL. 35 µL of antibody cocktail will be loaded into each reservoir.

1.3 Remove the chip from the wash buffer and dab the edge of the chip on a laboratory wipe to remove excess Wash Buffer. Be careful not to touch the gel surface to the laboratory wipe as it can damage or contaminate the gel.

1.4 Remove all free liquid from the chip by spinning it in a slide spinner for 3 seconds. Avoid any additional drying of the chip.

2. PLACE YOUR scWEST CHIP ON THE THREE-PLEX FIXTURE

2.1 Holding your chip gel side down, position the chip with the barcoded end touching the fixture outside reservoir A (**Figure 2**). Before lowering your chip, position it to leave a small gap between the bottom edge of the chip and the bottom edge of each antibody reservoir.

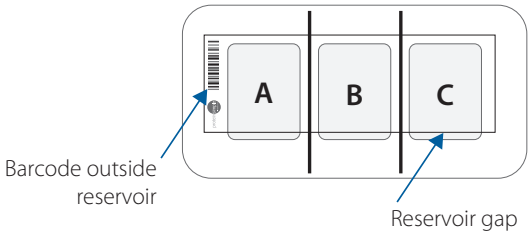

Figure 2. Proper alignment of an scWest chip on the Three-Plex Antibody Probing Fixture. Lay the chip down such that there is a small gap between the bottom edge of the chip and the bottom edge of each reservoir.

2.2 Starting with the barcoded end touching the fixture, slowly lower the scWest chip onto the antibody incubation chamber as if you were applying a coverslip (**Figure 3**).

Figure 3. Gently lower and stabilize the scWest chip (gel side down) using the flat edge of the tweezers (without gripping with the tweezers).

3. LOAD THE PRIMARY ANTIBODY COCKTAILS

3.1 Position the pipette tip around the midpoint of the first reservoir gap, such that it is at a <45° angle from the probing fixture surface (**Figure 4**). Be careful not to scratch the probing fixture or reservoir with your pipette tip.

3.2 Gently hold the edges of the chip to keep it in place while you introduce the antibody solution. Slowly (within 3–5 seconds) pipette 35 µL of your antibody cocktail into one of the outer reservoir gaps and allow the liquid to wick across the chip. Be careful not to introduce air bubbles, or apply excessive pressure/stress on the gel.

3.3 Load the other two reservoirs in the same way using the other antibody cocktail solutions. Where possible, load different species primaries in neighboring reservoirs and same species primaries in outermost, non-adjacent reservoirs. This will allow you to monitor if poor chip handling caused antibody leakage into neighboring chambers.

If incomplete loading is observed and you are loading all three chambers, gently nudge the chip with a pipette tip to eliminate the reservoir gap (see **Figure 2**). If loading only one chamber, leave the chip in place even if incomplete loading is observed to maintain gel integrity.

3.4 Incubate your chip with the antibody cocktails at room temperature for the desired time (typically 2 hours for primary antibodies).

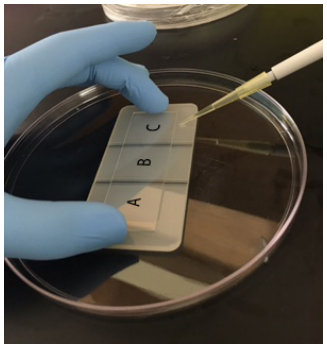

Figure 4. Example of proper placement of the pipette tip in the reservoir gap during reservoir filling.

4. REMOVE YOUR scWEST CHIP FROM THE FIXTURE

4.1 Lightly grip on the top (left and right) corners of the scWest chip with your thumb and index finger (**Figure 5**).

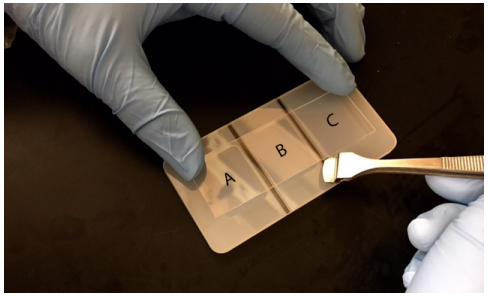

Figure 5. Removing the scWest chip from the probing fixture.

4.2 On the opposite side of the chip, gently insert the tweezers in the fixture groove between reservoirs B and C to carefully lever up the edge of the glass slide. Use care not to scratch the underlying probing fixture as repeated damage to the probing fixture can result in incomplete filling.

4.3 Lift up the chip and remove it from the fixture by pinching it between your thumb and index finger. Flip the chip so that it is gel-side up by rotating along the long edge in order to limit cross-contamination between the reservoirs.

4.4 Place the chip in a clean Petri dish with the gel facing up.

5. WASH THE scWEST CHIP

5.1 Pour 15 mL of 1X Wash Buffer into the Petri dish to cover the scWest chip. Place it on a shaker and wash it for 10 minutes.

5.2 Repeat two more times for a total of 3x10 minute washes.

6. CLEAN THE FIXTURE AND ADD SECONDARY ANTIBODY COCKTAILS

6.1 Rinse the fixture with DI water and air dry it (or if available) blow dry it with filtered air or nitrogen.

6.2 Repeat Steps 2–4 using your secondary antibody cocktail in place of your primary antibody cocktail. Note secondary antibodies are typically incubated for 1 hour at room temperature. Where possible, use secondary antibodies labeled with different spectral dyes in adjacent chambers to easily tell if poor chip handling caused antibody leakage into neighboring chambers, which would confound screening results.

7. WASH THE scWEST CHIP AND IMAGE

7.1 After incubating with secondary antibodies and placing your scWest chip gel face up in a Petri dish, 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.

7.2 Repeat two more times for a total of 3x15 minute washes.

7.3 Rinse the chip for 1 minute with DI water to remove salts before drying and imaging.

8. ANALYZE CHIP IMAGE IN SCOUT SOFTWARE

8.1 Launch Scout Software

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

8.3 Register each scanned image using auto or manual alignment (under the File menu).

8.4 Scout will automatically identify all the lanes in an image and all the peaks in each lane using default settings.

8.5 Reject regions of the chip located between each probing chamber region that were not probed with any antibody (visible as dark regions) by highlighting those regions, right clicking, and marking as "Rejected" [or keyboard shortcut "r"].

8.6 Optimize peak detection settings, exclude false positive peaks, label protein peaks of interest and visualize data as described in the Scout User Manual.

Important: Use Scout v2.0 or later to analyze images of scWest chips probed with a Three-Plex Antibody Probing Fixture.

TIPS AND TRICKS:

1. Careful introduction of antibody is important to prevent leakage between chambers. To visualize potential leakage, use different species primaries and alternate colors in adjacent chambers or probe for an internal control simultaneously and look for spatial variation in target expression in control-positive cells within the chamber.

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