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Staining of FFPE and Ac/EtOH-Fixed Tissue Sections Quick Guide

Below are the guidelines describing how to stain FFPE and Ac/EtOH-fixed tissue sections onto ZellSafe Tissue chips for ChipCytometry analysis.

Important Note on Quality Control for Tissue Stains

- It is strongly recommended to test all new antibodies on at least one or two positive control tissues of a matching preservation type (FF or FFPE). Antibodies might need a different dilution on target tissue than on positive control tissue.
- Make sure to check the initial scan images for tissue integrity (tissue detachment, folds and tissue damage) as well as high auto-FL. Compromised tissue integrity and high auto-FL can be a sign of low sample quality.
- Put a common biomarker that is expressed with high confidence in the target tissue in one of the early staining cycles. Check the early stain cycle images for signs of insufficient or unspecific staining, If one or more antibodies perform below expectations in terms of staining quality, this is an indication of insufficient sample quality.
- Perform the whole assay, including sample preparation, with a positive control tissue in parallel to the target tissues.
- On FFPE tissues, some biomarkers might require antigen retrieval conditions that are different from the standard conditions. When changing the antigen retrieval protocol make sure that all markers in your panel are compatible with the new conditions.

A. Staining with Conjugated Primary Antibodies

1. Place the chip with the labeled side up in the ZKW Washing station. Remove the sealing plug from the inlet of the ZellSafe™ chip. Pipette a few drops of ZKW wash buffer into the inlet to remove the air.
2. Plug the pipette adapter into the inlet of the ZellSafe™ chip and fill the adapter with ZKW wash buffer. Remove any air bubble from the pipette adapter by carefully aspirating the fluid.
3. Remove the sealing plug from the ZellSafe ™ chip outlet. Make sure that all air bubbles are removed and that a flow is established. Pipetting of all solutions should be done drop-by-drop.
4. Wash the chip with 1mL of ZKW wash buffer to remove the storage buffer from the chip.
5. Put the chip on the ZellScanner instrument and initiate photo bleach and background imaging.
6. Dilute one antibody or multiple antibodies as titrated in an appropriate volume of ZKW storage buffer. Mix by brief vortex. For ZellSafe Tissue Chips, use a total volume of 600µL.

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A. Staining with Conjugated Primary Antibodies Continued…

7. Pipette the antibody solution dropwise into the adapter. Take care not to introduce air bubbles into the chip.
8. Incubate for 15 minutes at room temperature on chips containing FF sections and 1 hour at room temperature for FFPE sections.
9. Wash with 1mL ZKW storage buffer.
10. Wash at least 3x with ZKW wash buffer for 5 minutes at each wash, for a total of 15mL, with a 2–5-minute pause between the wash steps. Repeat washing with 5mL ZKW wash buffer right before the scan if there was a longer time gap between the last wash cycle and the scan.
11. Scan on ZellScanner ONE. Repeat washing if back diffusion of antibody is observed during the scan.
12. Repeat A5-A11 until all desired markers have been stained.
13. For biomarker quantification on a single cell level staining with a DNA-binding dye is recommended. Dilute DNA dye in ZKW storage buffer as titrated, pipette into the chip and incubate 5-15 minutes at room temperature.
14. Follow the wash and scan procedure as described in A9-A11. Scan with the appropriate filter set.
    i. DAPI; FS395 & FS421
    ii. Hoechst®; FS395 & FS421
    iii. Propidium Iodide®; FS560

Note: DNA dyes are not photo-bleachable.

15. For chip storage, rinse the chip 2 times with 1 mL of sterile storage buffer.
16. Tightly seal the chip outlet with the sealing plug.
17. Remove the pipette adapter and seal the chip inlet.
18. Store chip at 4°C.

B. Staining with Primary and Secondary Antibody Pair

1. To avoid cross reactions with other antibodies, primary/secondary antibody staining must be applied in the first staining cycle(s). Make sure that primary antibodies in later cycles do not cross-react with the secondary antibodies present on the tissue. A blocking step with isotype control antibody solution might be necessary to avoid cross-reaction.
2. Put the chip on the ZellScanner and initiate photo bleach and background imaging.
3. Dilute the primary antibody in an appropriate volume of ZKW storage buffer. Mix by brief vortex. For ZellSafe Tissue Chips, use a total volume of 600µL.

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B. Staining with Primary and Secondary Antibody Pair Continued…

4. Pipette the primary antibody solution dropwise into the adapter. Take care not to introduce air bubbles into the chip.
5. Incubate for 15 minutes at room temperature on chips containing FF sections and 1 hour at room temperature for FFPE sections.
6. Wash with 1mL ZKW storage buffer, then wash with 5mL ZWK wash buffer.
7. Dilute the secondary antibody in an appropriate volume of ZKW storage buffer. Mix by brief vortex. For ZellSafe Tissue Chips, use a total volume of 600µL.
8. Pipette the secondary antibody solution into the chip and incubate for 10 minutes at room temperature on chips containing FF sections and 30 minutes at room temperature for FFPE sections.
9. Wash with 1mL ZKW storage buffer.
10. Wash at least 3x with ZKW wash buffer for 5 minutes at each wash, for a total of 15mL, with a 2–5-minute pause between the wash steps. Repeat washing with 5mL ZKW wash buffer right before the scan if there was a longer time gap between the last wash cycle and the scan.
11. Scan on ZellScanner ONE. Repeat washing if back diffusion of antibody is observed during the scan.

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