MOUSE LUNG IMMUNOFLUORESCENCE

IMPORTANT NOTE

This protocol has only been tested to work with Ki67, SP-C and CCA antibodies – other antibodies may require a modified antigen retrieval step and/or buffer.

EQUIPMENT REQUIRED

Slide racks and accessories
Heat resistant container to hold antigen retrieval buffer and slide racks
High humidity slide holder for antibody incubations
Electric pressure cooker for antigen retrieval
Pap pen
Paper towels
Glass slides and cover slips

REAGENTS REQUIRED

A. Antigen retrieval

- Antigen retrieval buffer: 0.1 M Tris-HCl, 5% w/v Urea, pH 9.5
- 1X PBS

B. First antibody

- Blocking buffer: 1X PBS, 2% w/v BSA, 0.3% w/v Triton-X 100
- Primary antibody diluted into Blocking buffer (SP-C/CCA - 1:200, Ki67 - 1:1000)
- Washing buffer: 1X PBS, 0.1% w/v Triton-X 100

C. Second antibody

- Blocking buffer: 1X PBS, 2% w/v BSA, 0.3% w/v Triton-X 100
- Secondary antibody diluted into Blocking buffer (Alexa fluorophores - 1:1000)
- Washing buffer: 1X PBS, 0.1% w/v Triton-X 100
- Washing buffer + DAPI (0.1 μg/mL final concentration)
- 1X PBS
- Aqueous anti-fade mounting medium (Prolong Gold Anti-Fade is good)

A. ANTIGEN RETRIEVAL

1 Place deparaffinized slides in a slide rack and submerge completely in antigen retrieval buffer (use a heat resistant container to hold the buffer). Place the submerged slides and container in a pressure cooker surrounded by an appropriate amount of water and heat for 10 minutes starting the timer only once the cooker is up to pressure.

2 After 10 minutes turn the pressure cooker off and allow it to cool down slowly for 30 minutes before removing the slides.

3 Dry the edges of each slide briefly with paper towel before applying the pap pen around the tissue (draw circles of increasing size for best results) before the tissue has time to dry out drip on an appropriate volume of 1X PBS within the circle of the pap pen to dilute out any remaining antigen retrieval buffer.

IMPORTANT NOTE
Always spin down buffers and antibody solutions at max speed for 5 minutes before using them on your tissue samples - this is to remove insoluble particulate matter and antibody complexes that may increase background.

B. First Antibody

1 Remove the 1X PBS.

2 Incubate at room temperature for 1 - 3 hours in blocking buffer.

3 Remove the blocking buffer and incubate overnight at 4 °C with primary antibody diluted appropriately in blocking buffer.

4 Wash 5 times with washing buffer (2 - 5 minutes for each wash is fine) to remove all traces of the primary antibody.

A. Second Antibody

1 Add the secondary antibody diluted appropriately in blocking buffer and incubate for 1 - 2 hour(s) at room temperature.

2 Wash once with washing buffer + DAPI for 10 minutes - this step removes most of the secondary antibody and stains the nuclei blue with DAPI at the same time.

3 Wash 3 times with washing buffer (2 – 5 minutes for each wash is fine) removing any remaining secondary antibody as well as the DAPI from the last step.

4 Wash once with 1X PBS for 2 – 5 minutes to remove any residual Triton-X 100 from the washing buffer.

5 Remove the 1X PBS and mount with anti-fade mounting medium before visualization.

IMPORTANT NOTE
After taking fluorescence images of your tissue samples it is possible to remove the cover slips and perform an H&E on the same slides. To remove the cover slips without disturbing the tissue architecture simply submerge the entire slide in water for 5 minutes at room temperature then proceed with the normal H&E protocol.

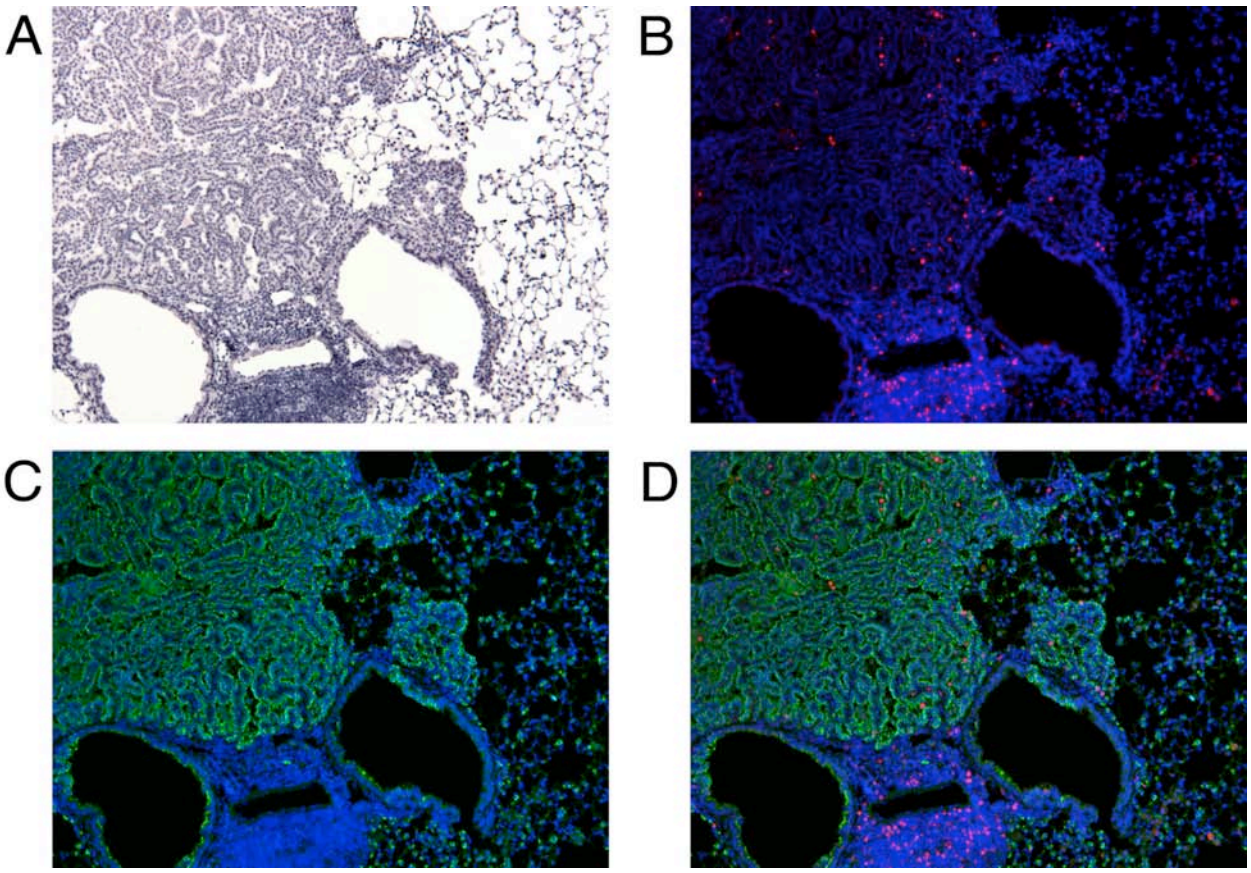

Figure 1. Example immunofluorescence image - A: H&E stain of mouse lung showing two distinct tumor types B: Ki67 (red) and DAPI (blue) overlay C: SP-C (green) and DAPI (blue) overlay D: Ki67 (red), SP-C (green) and DAPI (blue).