

## Miralys™ Protocol Practice Kit: Immunology Version

### Purpose

The Practice Kit was created to provide researchers with many of the materials needed to establish the Miralys™ Laboratory Workflow in their labs. Additionally, practice slides and probe mix enable initial Miralys™ runs without using up precious samples. This kit is to be used in conjunction with MALDI HiPLEX-IHC MIRALYS™ IMAGING LABORATORY WORKFLOW document (“Miralys™ Protocol”) which has been provided. If you do not have a copy, please contact [support@ambergen.com](mailto:support@ambergen.com) to obtain one.

### Contents

1. One (1) 6-plex Miralys™ probe mixture – store at -20°C and protect from prolonged light exposure. Details in table below.
2. Set of FFPE human tonsil slides – store at 4°C or at room temperature
  - a. One (1) “pre-stained” slide stained with the same 6-plex Miralys™ probe mixture detailed in item 1
  - b. Two (2) “unstained” slides sourced from the same tissue block as item (a)
3. One (1) multi-channel digital timer – for keeping time while working through the Miralys™ Protocol.
4. One (1) hydrophobic PAP barrier pen – used in Step 8 of the Miralys™ Protocol
5. Four (4) spin filter units (0.45 µm) – used in Step 10 of the Miralys™ Protocol
6. One (1) UV Lightbox with US and European power cords – used in Step 16 of the Miralys™ Protocol
7. Two (2) pieces of filter paper – used in Step 18 of the Miralys™ Protocol
8. One (1) glass petri dish (bottom only) – used in Step 18 of the Miralys™ Protocol
9. One (1) plastic petri dish – top only used in Step 18 of the Miralys™ Protocol
10. Four (4) neodymium magnets – used in Step 18 of the Miralys™ Protocol

## Probe Mix Details

Target	PC-MT (Da)*	Host	Reactivity	Final Concentration
CD3ε	1161.65	Rabbit	H	2.5 µg / mL
CD68	1216.75	Rabbit	H	2.5 µg / mL
Collagen-1A1	1234.87	Rabbit	M, H	2.5 µg / mL
Ki67	1320.76	Rabbit	H	2.5 µg / mL
PanCK	1628.78	Rabbit	M, R, H, Mk	2.5 µg / mL
VIM (Vimentin)	1230.84	Rabbit	M, R, H, Mk	2.5 µg / mL

\*PC-MT (Da) = Monoisotopic (M+H)<sup>+</sup> of the mass reporter

## Instructions for Use

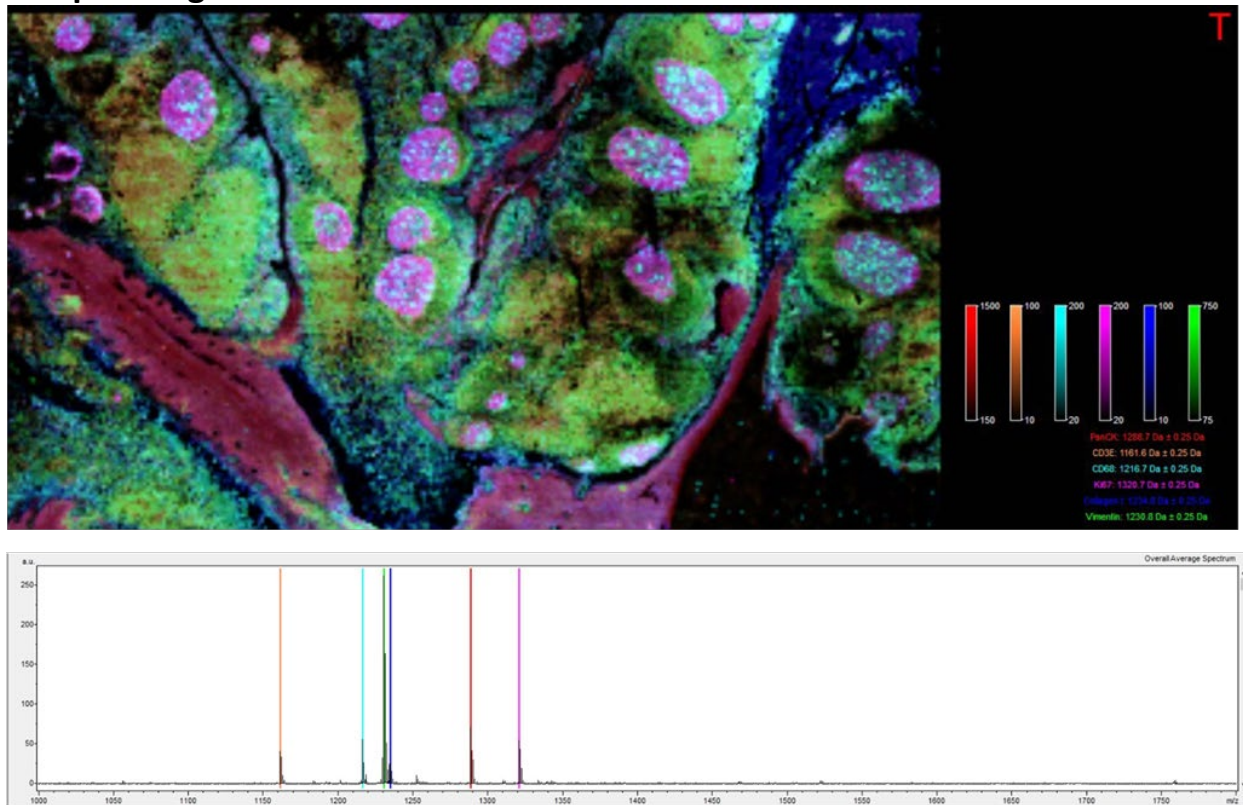
### Image the Pre-Stained Slide

1. Open the pink bubble-wrap slide envelope and the vacuum-sealed package within. Remove the pre-stained slide and re-store the unstained slides.  
**Important note: If slides were stored cold, equilibrate to room temperature before opening the vacuum-sealed pouch.**
2. Begin at Step 16 of the Miralys™ Protocol (photocleavage) and follow the protocol through to the end to prepare slide
3. Image in any MSI instrument

### Stain and Image the Unstained Slides

1. To prepare the unstained slides, begin by pre-melting as per Step 1 of the Miralys™ Protocol.
2. Complete Steps 2, 4, and 5 of the Miralys™ Protocol. *Step 3 can be omitted because the tissue is FFPE.*
3. Follow Steps 6 thru 8 of the Miralys™ Protocol, using alkaline antigen retrieval buffer in Step 6.
4. Because the Miralys™ probe is pre-mixed, perform the following in place of Step 9:
  - a. Prior to opening probe:
    - i. Vortex for 30 seconds with a benchtop vortex
    - ii. Centrifuge for 1 minute at full speed
  - b. For each slide, aliquot 24 µL of the supplied 48 µL probe mixture into a separate microcentrifuge tube and dilute to 400 µL final volume with the Tissue Blocking Buffer. Vortex for 30 seconds and centrifuge again for 1 minute.
5. Begin again with the Miralys™ Protocol at Step 10 and follow through to the end to prepare slides.
6. Image in any MSI instrument

## Sample Images



**Top:** Example Miralys™ Results from Pre-Stained FFPE Human Tonsil Tissue Slide. MALDI mass spectrometry imaging was performed on a Bruker rapifleX™ Tissue typer with 20 µm spatial resolution. The image was generated from flexImaging with TIC normalization and the display intensity scale in absolute units.

**Bottom:** Mean Spectrum from the Entire Region Shown. Color-coded bars indicate the reporter peaks from the photocleaved mass-tags (bars are color-coded according to the key provided in the image in the top panel).