Miralys™ Protocol Starter Kit: Neurology Version

Purpose

The Starter 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 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 mouse brain slides – store at 4°C or at room temperature
    a. One (1) "pre-stained" slide stained with the same 6-plex Miralys™ probe mixture as 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 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
GFAP	1011.55	Rabbit	M, R, H	2.5 µg / mL
GLUT1 (SLC2A1)	856.56	Rabbit	M, R, H, Mk	2.5 µg / mL
MBP (Myelin Basic Protein)	1365.73	Rabbit	M, R, H	1.25 µg / mL
NeuN	1308.71	Rabbit	M, R, H	2.5 µg / mL
NF-L (Neurofilament Light)	1345.74	Rabbit	M, R, H	1.25 µg / mL
SYN-I (Synapsin I)	1482.77	Rabbit	M, R, H	1.25 µg / mL

**PC-MT (Da) = Monoisotopic (M+H)+ of the mass reporter*

Instructions for Use

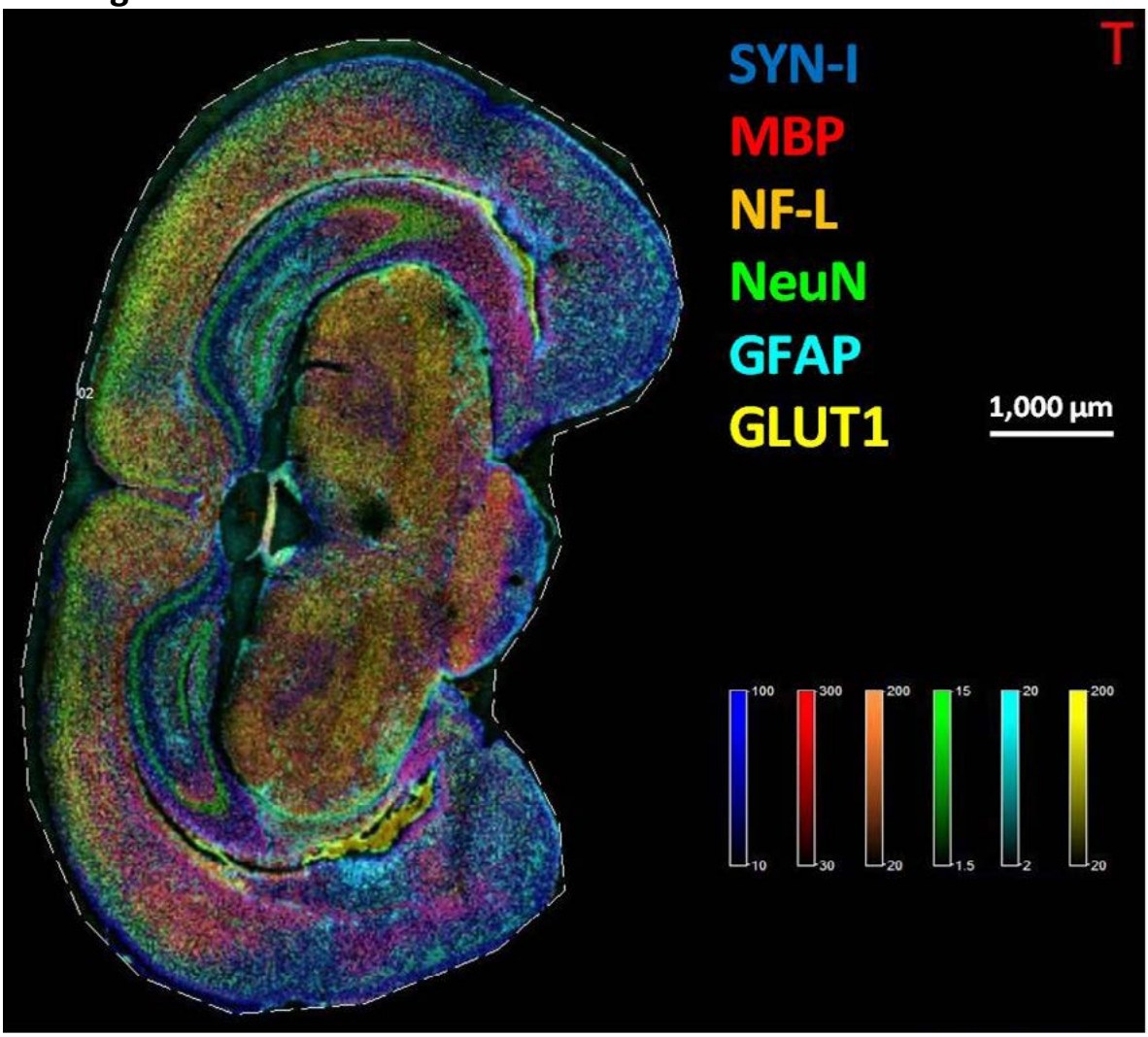
Image the Pre-Stained Slide

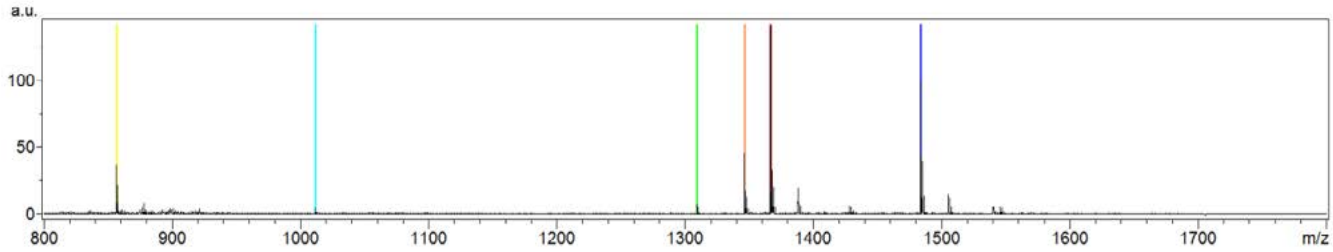
1. Open the pink bubble-wrap slide envelope and the vacuum-sealed package within. Remove the pre-stained slide and re-store the unstained slides.
   Important note: If slides were stored cold, equilibrate to room temperature before opening the vacuum-sealed pouch.
2. Begin at Step 16 of the Miralys™ 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 citrate 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 mouse brain tissue slide. MALDI mass spectrometry imaging was performed on a Bruker rapifleX™ Tissuetyper 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).

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