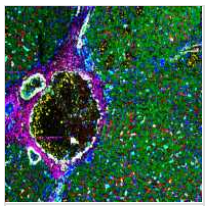

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Liver tissue staining with multiple lanthanides-tagged antibodies

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Liver Ln-Abs staining protocol

Hua Tian

Imaging mass spectrometry (IMS) is able to image the multiple lanthanides-tagged antibodies (up to 40) simultaneously at a subcellular resolution on a single tissue section. This protocol is developed for optimized immunostaining of frozen-hydrated tissue with antibodies cocktail (10-25 Abs), which produces consistent antibody affinity and improved IMS image quality using C60-secondary ion mass spectrometry (C60-SIMS).

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lanthanides-tagged antibody, immunostaining, imaging mass spectrometry, secondary ion mass spectrometry, C60-SIMS

protocol ,

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• Five micrometer fresh snap-frozen human liver sections on Au-coated Silicon wafer (2.2cm*2.2cm)
• 1% Paraformaldehyde (diluted from 16% solution from Thermo Fisher Scientific)
• Methanol (Sigma)
• 3% Goat serum (diluted from 10% stock from Thermo Fisher Scientific)
• DPBS (Corning)
• Wash buffer [DPBS supplemented with 0.05% Tween(Thermo Fisher Scientific) and 1% BSA(Sigma)]
• Metal-conjugated antibodies as in Table 1 below. Please refer to Fluidigm Maxpar Antibody Labeling User Guide Chapter 3 for detailed conjugation steps. Maxpar® X8 Antibody Labeling Kit with all the metal tags in Table 1 are purchased from Fluidigm

Table 1 Antibody panel for human liver tissue

A	B	C	D	E
Metal	**Target**	**Clone/Host**	**Cell/Pathway**	**Manufacturer/Catalog No.**
89Y	CD45	D9M8I, Rabbit IgG	Pan leukocyte	Cell Signaling/13917S
113 In	CD4	RPA-T4, mouse monoclonal	T cell	Novus/NBP2-25199
141Pr	SMA	1A4, Mouse IgG2a	Vascular walls/Hepatic Stellate cells/fibroblasts	Fluidigm/3141017D
143Nd	GFAP	EPR1034Y, Rabbit monoclonal	Ito Stellate Cells	Abcam/ab218309
145Nd	Heppar-1	HepPar1, mouse monoclonal	hepatocytes	Novus/NBP3-08970
147Sm	Glul	ab240193,Rabbit monoclonal	Pericentral hepatocytes (Zone 3)	Abcam/ab240193
148Nd	CD31	JC/70A, Mouse monoclonal	Endothelial cells	Abcam/ab264090
151Eu	CD68	D4B9C,Rabbit IgG	Macrophages	Cell Signaling/76437S
153Eu	CD32	FUN-2, Mouse IgG2b	Macrophages	Fluidigm/3153018B
158Gd	Arginase1	D4E3M™,Rabbit IgG	Zone 1-2 hepatocytes	Cell Signaling/93668S
161Dy	Albumin	EPR20195, Rabbit monoclonal	Periportal (Zone 1)	Abcam/Ab271979
166Er	CK19	SPM561, Mouse monoclonal	Cholangiocytes (Portal triad)	Abcam/ab212569

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168Er	Ki-67	Ki-67, Mouse IgG1	Proliferation (Midlobular)	Cell Signaling/9449S
169Tm	CD34	QBEND/10, Mouse / IgG1	Endothelial cells	Abcam/ ab198395
170 Er	EpCAM	E6V8Y, Rabbit IgG	Hepatic stem/progenitor cells	Cell Signaling /93790S
171Yb	LYVE1	EPR21857, Rabbit monoclonal	Sinusoidal endothelial cells	Abcam/ab232935
176Yb	EGFR	EP38Y, Rabbit monoclonal	cell membrane	Abcam/ ab272293
176Yb	Na/K ATPase	D4Y7E, Rabbit IgG	cell membrane	Cell Signaling/ 23565S
191Ir	Nuclear DNA		Nuclei	Fluidigm/ 201192B
196Pt	Human collagen I	EPR7785, Rabbit monoclonal	Collagen	Abcam/ab215969

- Intercalator-Ir (500µM, Fluidigm)
- DI water (Sigma)
- Water-Repellent Slides Marker Pen (Dando Sangyo Co Ltd. Japan)
- glass petri dish (diam 40mm, Sigma)

Wear proper PPE when handling the agents. Discard the waste in the biohazard waste container

1 Cut the fresh frozen sections at 5µm using a cyromicrotome and mount them on Au-coated silicon slides

2 Place the slides at -20 ◦C for 1h and then at 4 ◦C for 30 min for temperature equilibrium

3 Fix the tissue slides in 1% PFA for 5 min at 4 ◦C, then in prechilled methanol for 5 min at -20 ◦C

4 Wash the slides twice for 5 min in a glass petri dish by submerging the slides in 25 ml wash buffer, following by rehydrating the slides for 5 min in a glass container with 25ml DPBS and rinsing the slides for 5 min in a glass container with 25ml wash buffer

5 Use the Liquid-Repellent Slide Marker Pen to draw a circle around the tissue section to create

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a barrier to contain the solutions on the tissue sections

6 Apply 100 µl 3% goat serum solution to each slide for 1 h at RT and then remove excess block solution by tapping on a tissue

7 Prepare the antibody cocktail. Vortex all the antibodies for 15 s and then centrifuge at 12,000G for 2 min, followed by diluting the upper liquid of each antibody in 0.5% PBS as desired concentration

8 Add 100 µl of the antibody cocktail to each section and incubate overnight at 4◦C in a sealed glass petri dish in a fridge

9 After the incubation, wash the sections two times for 5min in a glass petri dish with 5 ml wash buffer with slow agitation

10 Incubate the slides with 100 µl 1:100 dilution of Intercalator-Ir in DPBS for 30 min at RT

11 After nuclei staining, wash the slides for 5 min in a glass container with 5 ml DPBS

12 Quickly dip the slides in a container with 5 ml DI water for 15 s, repeat three times

13 Dry the slides in a fume hood at RT for 20 min

14 Store the slides at 4◦C until C60-SIMS imaging.

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