

3D Visualization of Immune Cell Populations in HIV-Infected Tissues via Clearing, Immunostaining, Confocal, and Light Sheet Fluorescence Microscopy

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Materials

Name	Company	Catalog Number	Comments
Acrylamide Solution (in 0.1 M PBS, 40 mL in total)			
40% Acrylamide: 4 mL	Bio-Rad	1610144	
VA-044 Thermal Initiator: 0.1g	Fujifilm	011-19365	
CLARITY Blocking solution (in 0.1 M PBS, 5 mL in total)			
Fetal bovine serum (FBS): 200 µL	Atlas Biologicals	F-0500-D	
Rat anti-human or anti-mouse FcR: 50 µL	Miltenyi	130-092-575(mouse)/130-059-901(human)	
Sodium azide (from stock solution): 5 µL	Sigma	71289-50G	
Tween-20: 5 µL	Fisher Scientific	BP337-500	
CLARITY wash solution (in 0.1M PBS, 50 mL in total)			
Sodium azide (from stock solution): 50 µL	Sigma	71289-50G	
Tween-20: 50 µL	Fisher Scientific	BP337-500	
CUBIC Blocking solution (in 0.1M PBS, 5 mL in total)			
Fetal bovine serum (FBS): 200 µL	Atlas Biologicals	F-0500-D	
Rat anti-human or anti-mouse FcR: 50 µL	Miltenyi	130-092-575(mouse)/130-059-901(human)	
Sodium azide (from stock solution): 5 µL	Sigma	71289-50G	
Triton X-100: 5 µL	VWR	M143-1L	
CUBIC Reagent-1 (in 0.1M PBS, 50 mL in total)			

N, N, N', N'-tetrakis (2-hydroxypropyl) ethylenediamine: 12.5 g	Aldrich	122262	
Triton X-100: 7.5 g	VWR	M143-1L	
Urea: 12.5 g	Fisher chemical	U15-500	
CUBIC Reagent-2 (in 0.1M PBS, 50 mL in total)			
Sucrose: 25 g	Sigma	S1888-500G	
Sodium azide (in powder form): 10 g	Sigma	71289-50G	
Sodium azide stock solution (in DI H ₂ O, 50 mL in total)	Sigma	71289-50G	
Triethanolamine: 5 g	Sigma	90270-500mL	
Triton X-100: 50 µL	VWR	M143-1L	
Urea: 12.5 g	Fisher chemical	U15-500	
CUBIC wash solution (in 0.1M PBS, 50 mL in total)			
Sodium azide (from stock solution): 50 µL	Sigma	71289-50G	
Triton X-100: 50 µL	VWR	M143-1L	
DAPI staining solution (0.5 µg/mL)			
DAPI stock solution: 1 µL			
Wash solution: 10 mL			
DAPI stock solution (5 mg/mL)			
DAPI powder: 5 mg	Sigma-Aldrich	D9542-1MG	
DMSO (100%): 1 mL	ThermoFisher	D12345	
Imaging Media RI-2 (in dH₂O)			
90% Histodenz	Sigma	D2158-100G	
0.01% Sodium azide	Sigma	71289-50G	
0.02 Sodium Phosphate Buffer, pH 7.5	Sigma-Aldrich	S9638-250G	
0.1% Tween-20	Fisher Scientific	BP337-500	
Primary antibodies (in blocking solution without rat anti-mouse FcR, 2 mL in total)			
Goat anti-HIV p24: 10 µL (1:200)	Creative Diagnostics	DPATB-H81692	
Mouse anti-human CD68: 10 µL(1:200)	Dako	M0876	
Rabbit anti-human CD3: 10 µL (1:200)	Dako	A0452	
8% SDS Solution (in 0.1 M PBS, 50 mL in total)			
SDS powder: 4 g	Sigma-Aldrich	L3771-500G	
Secondary antibodies (in blocking solution without rat anti-mouse FcR, 2 mL in total)			
Donkey anti-goat conjugated with AlexaFluor647: 2 µL	Invitrogen	A21447	
Donkey anti-mouse conjugated with AlexaFluor594: 2 µL	Invitrogen	A21203	
Donkey anti-rabbit conjugated with AlexaFluor488: 2 µL	Invitrogen	A21206	