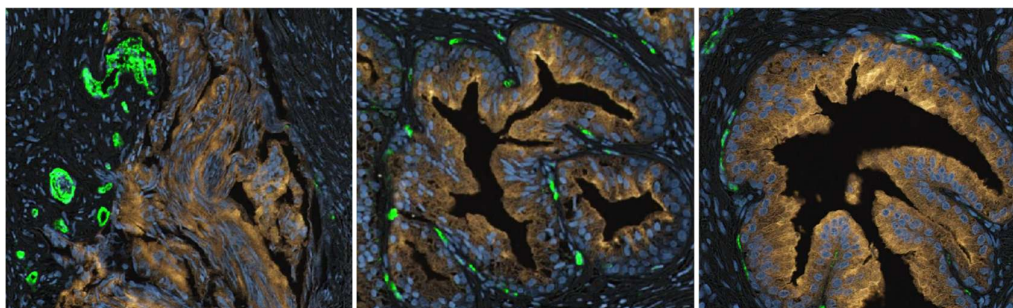


CD31 and PSMA Duplex Labelling with SCIZYS Erbium and HRP-DAB



Human prostate tissue; green (SCIZYS Erbium-SA): CD31; brown (DAB): PSMA

Material	Supplier	Article #
Monoclonal rabbit anti-PSMA antibody	Abcam	ab133579
Polyclonal mouse anti-CD31 antibody	Abcam	Ab199012
EnVision FLEX Target Retrieval Solution High pH (50x), Concentrate	Agilent/Dako	K800421-2
Xylene	HistoLab	AS-GPS1001-G
Absolute Ethanol	HistoLab	01399.05L
Mayers Haematoxylin	HistoLab	01820
SuperFrost Microscope Slides	HistoLab	06340X40
Coverslips #1.5 24×50 mm	HistoLab	06832
Mounting Medium Pertex	HistoLab	00840-05
Phosphate-buffered saline (PBS)	In-house	-
Biotin-SP AffiniPure goat Anti-Rabbit IgG	Jackson Immunoresearch	111-065-144
SCIZYS Dilution Buffer	Lumito	SZDB-01-01
SCIZYS Erbium-SA	Lumito	SZERSA-01-01
SCIZYS Wash Buffer (20×)	Lumito	SZWB-01-01
3,3'-Diaminobenzidine tetrahydrochloride hydrate (DAB)	Merck	D5637
Hydrogen peroxide 30%	Merck	1.07209
ProLong Diamond Antifade Mountant	Thermo Fisher Scientific	P36961
Goat Anti-Mouse IgG Antibody (H+L), Biotinylated	Vector Laboratories	BA-9200-1.5
ImmPRESS HRP Goat Anti-Rabbit IgG Polymer Detection Kit, Peroxidase	Vector Laboratories	MP-7451
ImmEdge Hydrophobic Barrier PAP pen	Vector Laboratories	H-4000
Normal Goat Serum Blocking Solution, 2.5%	Vector Laboratories	S-1012-50
Streptavidin/Biotin Blocking Kit	Vector Laboratories	SP-2002

IHC Labelling Protocol

Sections: 3–4 µm on SuperFrost microscope slides

Pre-treatment: bake slides 1 h at 60 °C

1. Dilute SCIZYS wash buffer (20×) with deionized water (dH₂O), for 1 L of 1× buffer add 50 mL of stock buffer to 950 mL dH₂O
2. Prepare HIER cuvette with HIER buffer and put into water bath set to 95 °C
3. Xylene 2×5 min
4. Absolute ethanol (EtOH) 2×5 min
5. 96% EtOH 5 min
6. 70% EtOH 5 min
7. dH₂O 5 min
8. HIER: 20 min at 95 °C
9. Cool at room temperature 20 min
10. dH₂O 1 min
11. Mayers Haematoxylin 3 min
12. Remove excess haematoxylin with dH₂O
13. SCIZYS Wash Buffer 1 min
14. 0.3% H₂O₂ in PBS 10 min
15. SCIZYS wash buffer 1 min
16. Encircle section with silicone pen
17. SCIZYS wash buffer 1 min
18. 2.5% goat serum 20 min
19. SCIZYS Wash Buffer 1 min
20. Streptavidin solution 20 min
21. SCIZYS Wash Buffer 2× 1 min
22. Biotin solution 20 min
23. SCIZYS Wash Buffer 2× 1 min
24. Antibody cocktail of Anti-PSMA antibody (1:400 diluted) + anti-CD31 antibody (2 µg/mL) 90 min
25. SCIZYS Wash Buffer 2× 1 min
26. Biotinylated anti-mouse antibody (2 µg/mL in SCIZYS Dilution Buffer) 30 min
27. SCIZYS Wash Buffer 3× 1 min
28. SCIZYS Erbium-SA (50 µg/mL diluted in ready-to-use goat anti-rabbit IgG polymer detection solution) 30 min
29. SCIZYS Wash Buffer 3× 1 min
30. Substrate mix (0.5 mg/mL DAB, 0.1% H₂O₂ in PBS) 5 min (room temperature, dark)
31. SCIZYS Wash Buffer 1 min
32. Dip in dH₂O
33. Dry on air
34. Mount and coverslip with Pertex