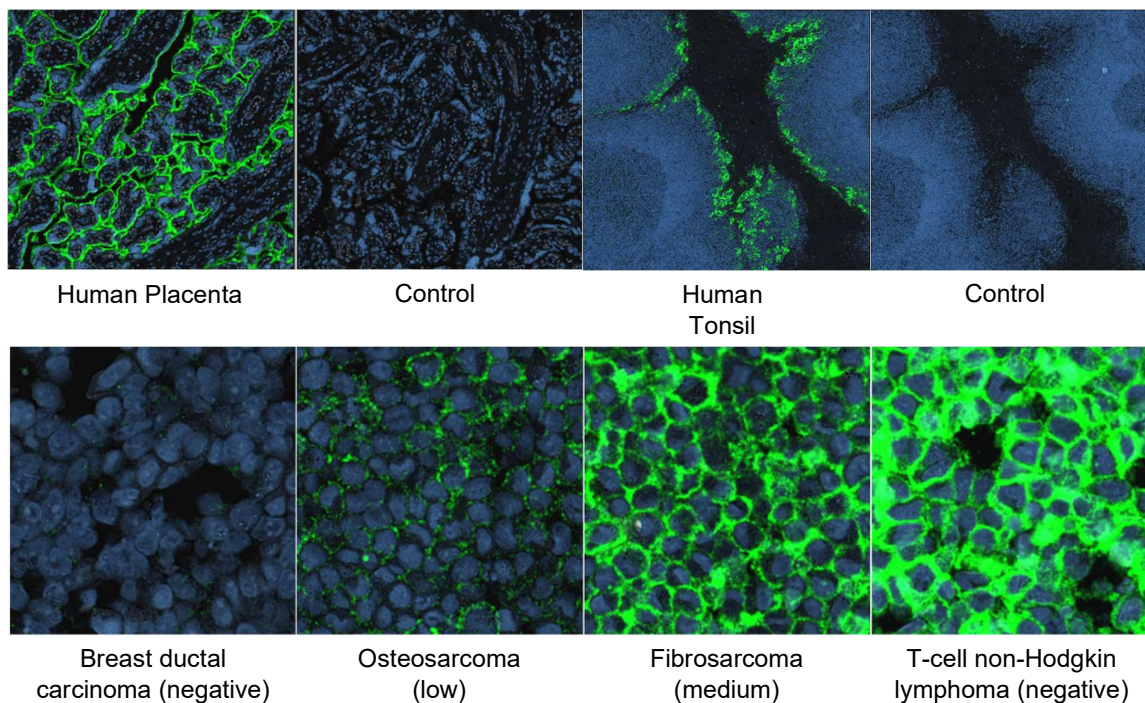


PD-L1 Labelling with SCIZYS Erbium



Material	Supplier	Article #
EnVision FLEX Target Retrieval Solution High pH (50×), Concentrate	Agilent/Dako	K800421-2
Xylene	HistoLab	AS-GPS1001-G
Absolut Ethanol	HistoLab	01399.05L
Mayers Haematoxylin	HistoLab	01820
SuperFrost Microscope Slides	HistoLab	06340X40
Coverslips #1.5 24×50 mm	HistoLab	06832
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
ProLong Diamond Antifade Mountant	Thermo Fisher Scientific	P36961
Monoclonal rabbit anti-PD-L1 antibody	Validated Antibodies	MSVA-711R
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 distilled 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. Encircle section with silicone pen
14. SCIZYS wash buffer 1 min
15. 2.5% goat serum 20 min
16. SCIZYS Wash Buffer 1 min
17. Streptavidin solution 20 min
18. SCIZYS Wash Buffer 2× 1 min
19. Biotin solution 20 min
20. SCIZYS Wash Buffer 2× 1 min
21. Anti-PD-L1 antibody (1:150 diluted in SCIZYS Dilution Buffer) 90 min
22. SCIZYS Wash Buffer 2× 1 min
23. Biotinylated anti-rabbit antibody (2 µg/mL in SCIZYS Dilution Buffer) 30 min
24. SCIZYS Wash Buffer 2× 1 min
25. SCIZYS Erbium-SA (50 µg/mL in SCIZYS Dilution Buffer) 30 min
26. SCIZYS Wash Buffer 3× 1 min
27. Mount and coverslip with ProLong Diamond Antifade