

Materials List for:

# Four-color Fluorescence Immunohistochemistry of T-cell Subpopulations in Archival Formalin-fixed, Paraffin-embedded Human Oropharyngeal Squamous Cell Carcinoma Samples

Simone Punt<sup>1</sup>, Robert J. Baatenburg de Jong<sup>2</sup>, Ekaterina S. Jordanova<sup>1,3</sup>

<sup>1</sup>Department of Pathology, Leiden University Medical Center

<sup>2</sup>Department of Otorhinolaryngology and Head and Neck Surgery, Erasmus University Medical Center

<sup>3</sup>Center for Gynecological Oncology Amsterdam, VU Medical Center

Correspondence to: Simone Punt at [S.vd.Garde@outlook.com](mailto:S.vd.Garde@outlook.com)

URL: <https://www.jove.com/video/55589>

DOI: [doi:10.3791/55589](https://doi.org/10.3791/55589)

## Materials

Name	Company	Catalog Number	Comments
Pathos Delta Ultra Rapid Tissue Processor	Milestone and Histostar		Automated tissue processor
Histostar	Thermo Scientific		Tissue block embedding machine
Formaldehyde	Baker		
Xylo	Merck		
Ethanol	Merck		
milliQ water	Elaga Purelab Chorus		
Paraffin wax/Paraclean	Klinipath	5079A	
Microtome tissue holder	Leica	RM225	
Flex IHC slide	Dako		Tissue slide
Tris	Merk-Milipore	1,083,821,000	
EDTA	Baker	1073	
PBS	Bio-Rad	BUF036A	
BSA	Sigma	A9647	
Rabbit anti-CD3	Abcam	ab828	Titrate required antibody dilution
Mouse IgG1 anti-FoxP3	Abcam	ab20034	Titrate required antibody dilution
Goat IgG anti-IL-17	R&D Systems	AF-317-NA	Titrate required antibody dilution
Rabbit Ig isotype control antibody	Abcam	ab27472	Use at same final concentration as anti-CD3
Mouse IgG1 isotype control antibody	Abcam	ab91353	Use at same final concentration as anti-FoxP3
Goat IgG isotype control antibody	ThermoFisher Scientific	02-6202	Use at same final concentration as anti-IL-17
Donkey anti-rabbit IgG A546	ThermoFisher Scientific	A10040	Dilute 1:200 in 1% BSA/PBS
Donkey anti-mouse-A647	ThermoFisher Scientific	A31571	Dilute 1:200 in 1% BSA/PBS
Donkey anti-goat IgG A488	ThermoFisher Scientific	A11055	Dilute 1:200 in 1% BSA/PBS
VectaShield containing DAPI	Vector Laboratories	H-1200	
LSM700 confocal laser scanning microscope	Zeiss		
LCI Plan-Neofluar 25x/0.8 Imm Korr DIC M27 objective	Zeiss	420852-9972-720	
LSM Zen Software	Zeiss	version 2009	
LSM Image Browser	Zeiss	version 4.2.0.121	Available to download at <a href="http://www.zeiss.com/microscopy/int/">www.zeiss.com/microscopy/int/</a>

			website/downloads/lsm-image-browser.html.
SPSS	IBM Corp.	version 20.0	
ImageJ		version 1.50i	Available to download at <a href="http://rsb.info.nih.gov/ij">http://rsb.info.nih.gov/ij</a> .