

Online resource 1: Immunohistochemical staining technique used for staining of FOXP3, CD25/CD4

For IHC staining, appropriately labelled sections on Poly L lysine coated slides were dewaxed by placing them on hot plate for 56C for 5 minutes, deparaffinized in xylene and brought through descending concentration of alcohol and water. For antigen retrieval, the slides were immersed in TRIS EDTA (pH 9.0) kept in trough and the trough was kept in decloaker (retrieval) till it reaches the temperature of 125 for 15 minutes. Sections were washed with three changes of Tris wash buffer (pH 7.6) for 5 minutes each. Endogenous peroxide blocking was done by adding 1 drop of 3% H₂O₂ to the sections and incubated for 10 minutes in moist chamber. Sections were washed with three changes of Tris wash buffer (pH 7.6) for 3 minutes each and incubated in background sniper solution for 5-10minutes.

Dilution and primary antibodies [GenomeMe FoxP3 antibody (IHC773) and CD25(RBT-CD25), RMab- Bio-SB, CD4 antibody] were put on slide for overnight at 4C. Sections were washed with three changes of Tris wash buffer (pH 7.6) for 3 minutes each and incubated in super enhancer for 30 minutes. Sections were washed with three changes of Tris wash buffer (pH 7.6) for 3 minutes each and incubated in polymer HRP antibody for 30 minutes. Sections were again washed with three changes of Tris buffer (pH 7.6) for 5 minutes each. For CD4 staining: pH 6.0 citrate buffer was used for antigen retrieval. Freshly prepared DAB solution [BioGenex SS polymer HRP+DAB] was added to section and stain controlled microscopically. Sections were then washed in distilled water for 5 minutes and counterstaining done by haematoxylin for 1-2 minutes. Blueing was done under tap water and sections were dehydrated, cleared and mounted in DPX.