

Immunofluorescence: Mouse Anti-Human RANTES Protocol

The following protocol used formalin-fixed paraffin-embedded human tonsil tissue.

1. Deparaffinize and rehydrate the tissue section.
2. Perform heat-induced antigen retrieval with 0.1M citrate buffer pH 6.0 at 123°C for 3 minutes in a pressure chamber. Wash the slide twice for three minutes each (50mM Tris-HCl pH 8.0, 150mM NaCl in distilled water).
3. Incubate the tissue section with 1% BSA/PBS block.
4. Incubate the tissue section overnight at 4°C with Mouse Anti-Human RANTES at 10.0 µg/mL. Wash the slide twice for three minutes each.
5. Incubate the tissue section with an HRP polymer kit according to the manufacturer's protocol. Wash the slide twice for three minutes each.
6. Incubate the tissue section with the DAB chromogen.
7. Counterstain the tissue section with hematoxylin.
8. Coverslip the sections with mounting medium.

**Protocol and staining provided by Dr. Lauren Binge, Laboratory of Prof. Charles Mackay, Monash University, Australia*