

Immunofluorescence: Mouse Anti-Human MCP-1 Protocol

Immunohistochemistry protocol for Mouse Anti-Human MCP-1*:

The following protocol used formalin-fixed paraffin-embedded human tonsil tissue (supplied by the Alfred Hospital, Australia).

1. Deparaffinize and rehydrate the tissue section.
2. Perform heat-induced antigen retrieval with 1mM EDTA pH 8.0 at 123°C for 3 minutes in a Pascal pressure chamber (Dako). 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 MCP-1 at 10.0 µg/mL. Wash the slide twice for three minutes each.
5. Incubate the tissue section with the Envision+ HRP polymer kit (Dako) 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 DPX mounting medium and image sections with an Olympus Provis light microscope (Monash Micro Imaging, Monash University, Australia).

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