Immunofluorescence: Rabbit Anti-Murine RELMα Protocol

The following protocol used B6 mice that were infected with 200 embryonated Trichuris muris eggs and sacrificed 15 days post-infection. The tip of the cecum was removed, rinsed in 1X PBS pH 7.2, and fixed in 4% PFA. Following fixation, the tissue was embedded in paraffin and cut into 5 µM sections.

1. Deparaffinize and rehydrate the tissue section.
2. Perform heat-induced antigen retrieval by boiling the tissue section in 10mM pH 6.0 citrate buffer for 25 minutes.
3. Incubate the tissue section with blocking buffer for 20 minutes.
4. Incubate the tissue section overnight at 4°C with Rabbit Anti-Murine RELMα at 4.0 ng/mL in 1X PBS with 0.01% Triton-X and 0.5% BSA. Wash the slide twice for three minutes (1X PBS/0.05% Tween 20).
5. Incubate the tissue section with a fluorescent conjugated secondary antibody for 2 hours at room temperature. Wash the slide twice for three minutes.
6. Counterstain the tissue section with DAPI.

The following protocol used B6 mice lung sections that were injected with helminth Schistosoma mansoni eggs. Inflamed lung sections were fixed in 4% PFA. Following fixation, the tissue was embedded in paraffin and cut into 5 µM sections. Some tissue sections or cell preparations stained were from day 14 bleomycin-instilled mice.

1. Deparaffinize and rehydrate the tissue section.
2. Perform heat-induced antigen retrieval by boiling the tissue section in 10mM pH 6.0 citrate buffer for 25 minutes.
3. Incubate the tissue section with blocking buffer for 20 minutes.
4. Incubate the tissue section overnight at 4°C with Rabbit Anti-Murine RELMα at 0.2 µg/mL in 1X PBS with 0.01% Triton-X and 0.5% BSA. Wash the slide twice for three minutes (1X PBS/0.05% Tween 20).
5. Incubate the tissue section with a fluorescent conjugated secondary antibody for 2 hours at room temperature. Wash the slide twice for three minutes.
6. Counterstain the tissue section with DAPI.

**Information and photo are courtesy of David Artis, University of Pennsylvania. Please refer to the following references:*

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