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Immunofluorescence: Rabbit Anti-Murine RELMβ Protocol

The following protocol used B6 mice that were infected with 200 embryonated Trichuris muris eggs and sacrificed 21 days post-infection. The tip of the cecum was removed, rinsed in PBS, 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 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 RELMβ at 0.2 μg/mL in 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:

Goblet cell-derived resistin-like molecule beta augments CD4+ T cell production of IFN-gamma and infection-induced intestinal inflammation.Nair MG, Guild KJ, Du Y, Zaph C, Yancopoulos GD, Valenzuela DM, Murphy A, Stevens S, Karow M, Artis D. J Immunol. 2008 Oct 1;181(7):4709-15.

Novel effector molecules in type 2 inflammation: lessons drawn from helminth infection and allergy. Nair MG, Guild KJ, Artis D. J Immunol. 2006 Aug 1;177(3):1393-9.

RELMbeta/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. Artis D, Wang ML, Keilbaugh SA, He W, Brenes M, Swain GP, Knight PA, Donaldson DD, Lazar MA, Miller HR, Schad GA, Scott P, Wu GD. Proc Natl Acad Sci U S A. 2004 Sep 14;101(37):13596-600.

TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. Taylor BC, Zaph C, Troy AE, Du Y, Guild KJ, Comeau MR, Artis D. J Exp Med. 2009 Mar 9.