Immunostaining of the isolated lymphoid follicles (ILFs) in the whole small intestine Version 2

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Abstract

The immunostaining detection of isolated lymphoid follicles (ILFs) was performed according to the method of McDonald and Newberry [BioTechniques. 2007; 43(1): 50-56], with some modifications. Instead of mounting the tissue samples on a mounting plate, each segment was treated in a 15 ml tube in each step. DAB stained intestines were finally mounted on a glass slide using an aqueous mounting medium.

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Materials

Hanks' Balanced Salt solution, 10 ×, Modified, without calcium, magnesium or sodium bicarbonate H4385-100ML by Sigma Aldrich

Avidin/Biotin Blocking Kit SP-2001 by Vector Laboratories

Purified Rat Anti-Mouse CXCR5 551961 by BD Biosciences

Anti-Rat Ig HRP detection kit 551013 by BD Biosciences

✓ Mount-Quick Aqueous View by Contributed by users

Protocol

Preparation

Step 1.

Open small intestine vertically

Step 2.

Rince the intestine with cold PBS in petri dish

Step 3.

Divide a small intestine into four segments (A to D, proximal to distal).

Step 4.

Place each segment into a 15 ml tube filled with 10 ml warmed (37℃) Hank’s balanced salt solution (HBSS) containing 5 mM EDTA at 37℃

Removal of the epithelial cells

Step 5.

Incubate tubes on shaker at 37℃ for 15 min (shaking 90 rmp) and then shake the tubes vigorously 20 times by hand.

Step 6.

Discard supernatant containing epithelium and filter remaning the intestinal segment through a gauze.

Step 7.

Put retained the intestinal segment in the 15 ml tube containing 10 ml HBSS containing 5 mM EDTA.

Step 8.

Repeat steps 5-7 for 3 times.

Fixation

Step 9.

Rince the segment with cold PBS and submerge the segment in 10% formalin-buffered saline and place at 4℃ at least 1h.

Step 10.

Wash the segment for 5 min with 50 mM Tris-HCl (pH 7.2) buffered saline (150 mM NaCl) (TBS) containing 0.5% Triton X-100 for 3 times.

Quenching

Step 11.

Add 1% H_2O_2 in methanol and shake 15 min at room temperature (rt).

Step 12.

Wash for 5 min with TBS containing 0.05% Tween 20 (TBST) for 3 times.

Blocking

Step 13.

Add 1 drop Avidin D solution (Avidin/Biotin Blocking Kit) in 250 µl PBS containing 1% BSA and incubate 30 min at rt.

Then wash the segment with TBS.

Primary antibody (anti-CXCR5)

Step 14.

Add 1 drop of biotin solution (Avidin/Biotin Blocking Kit) and 2.5 µl of primary antibody* in 250 µl TBST (* Rat anti-mouse CXCR5 (clone 2G8, BD Biosciences, San Diego, CA) and incubate overnight at 4℃.

Wash the segments for 5 min with PBS 3 times.

Secondary antibody (Anti Rat Ig HRP)

Step 15.

Add 2 µl biotinylated anti-Ig secondary antibody□Anti-Rat Ig HRP detection kit, Pharmingen) in 400 µl antibody diluent and incubate for 30 min at rt.

Wash for 2 min with PBS 3 times.

Step 16.

Add 350 µl Streptavidin-HRP and incubate for 30min at rt.

Wash for 2 min with PBS 3 times.

Detection

Step 17.

Drain PBS. Add 300 µl 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (1 drop DAB/1ml DAB buffer).

Incubate for 5 min or until the desired color intensity is reached.

Wash for 2 min with PBS 3 times.

Rince the segment with water for 2 min 3 times

Mounting

Step 18.

Mount the stained segments of intestine on a slide glass and fixed with Mount-Quick Aqueous.