Copy of Immunohistochemistry

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Zhang N, Gao P, Yin B, Li J, Wu T, Kuang Y, Wu W, Li J (2019) Cathepsin L promotes secretory IgA response by participating in antigen presentation pathways during *Mycoplasma Hyopneumoniae* infection. PLoS ONE 14(4): e0215408. doi: 10.1371/journal.pone.0215408

MATERIALS

NAME	CATALOG #	VENDOR
0.1% Triton X-100-containing 1XPBS solution/5% normal goat serum		
Sodium phosphate dibasic	7558-79-4	Sigma Aldrich
Tween-20	P-7949	Sigma-aldrich
Sensitive DAB Stain Kit	PW023.SIZE.5Preps	Bio Basic Inc.
EDTA	AM9261	Invitrogen - Thermo Fisher
Antibody Diluent OP Quanto	TA-125-ADQ	Thermo Fisher Scientific
Tris Buffered Saline & Tween 20 (20x)	TA-999-TT	Thermo Fisher Scientific

1 Deparaffinize/hydrate sections: a.Incubate sections in three washes of xylene for 5 minutes each.b.Incubate sections in two washes of 100% ethanol for 10 minutes each.c.Incubate sections in two washes of 95% ethanol for 10 minutes each.

2 Wash sections twice in dH2O for 5 minutes each.

3 Incubate sections in 3% hydrogen peroxide for 10 minutes.

4 Wash sections in wash buffer for 5 minutes.

5 Block each section with 100-400 µl blocking solution for 1 hour at room temperature.

6 Remove blocking solution and add 100-400 µl primary antibody diluted in recommended antibody diluent to each section. Incubate overnight at 4°C.

7 Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.

8 Add 100-400 µl biotinylated secondary antibody, diluted in TBST per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.

9 If using ABC avidin/biotin method, prepare ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.

10 Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.

11 Add 100-400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.

12 Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.

13 Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.

14 As soon as the sections develop, immerse slides in dH2O.

15 If desired, counterstain sections in hematoxylin per manufacturer's instructions.

16 Wash sections in dH2O two times for 5 minutes each.

17 Dehydrate sections: a.Incubate sections in 95% ethanol two times for 10 seconds each.b.Repeat in 100% ethanol, incubating sections two times for 10 seconds each.c.Repeat in xylene, incubating sections two times for 10 seconds each.

18 Mount coverslips.