# Immunoprecipitation assay

PLOS One

Ning Zhang , Peng Gao[1]

[1]China Agricultural University

dx.doi.org/10.17504/protocols.io.zgbf3sn

Apr 16, 2019

Working

China Agricultural University

Tech. support email: **annachuang@cau.edu.cn**

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0215408

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

GUIDELINES

IP-1.jpg IP-2.jpg

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| PBS | | Invitrogen - Thermo Fisher |
| Protein A/G Agarose | | Santa Cruz Biotechnology |
| NP-40 | | Sigma Aldrich |
| MHC class II antibody | K274.3G8 | Abcam |

1 To harvest cells in nondenaturing conditions, remove media and rinse cells with ice cold 1×PBS

2 Remove PBS and add 0.5 ml ice cold cell lysis buffer to each plate(10cm) and incubate on ice for 5 min

3 native protein: Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice. Sonicate on ice 3 times for 5 sec each. [denatured condition: scrape cells off the plate and transfer to 2.5 ml tube, boil for 10 min, then add 4 volumes cell lysis buffer, this is the cell lysate. ]

4 Native protein : Microcentrifuge for 10 min at 4 °C,14000g and transfer the supernatant to a new tube. The supernatant is the cell lysates, store at -80°C.

5 add primary antibody(at the appropriate dilution as recommended in the product datasheet) to 200ul cell lysate at 1mg/ml. incubate with gentle rocking overnight at 4°C.

6 Add protein A/G agarose beads (10-30ul of 50% bead slurry). Incubate with gentle rocking for 1-3h at 4 °C.

7 Microcentrifuge for 30 sec at 4°C. Wash pellet five times with 500ul cell lysis buffer. Keep on ice between washes.

8 Resuspend the pellet with 20ul SDS sample buffer. Vortex, then microcentrifuge for 30sec.

9 Heat the sample to 95-100°C fro 5 min and microcentrifuge for 1min at 14000g.Or, do not heat.

10 Load the sample on a 4-20% gel for SDS-PAGE.

11 Analyze sample by western blotting.