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We use this protocol and it's working

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Endogenous coimmunoprecipitation

In 2 collections

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ABSTRACT

Protocol used for immunoprecipitation of HSP60 and LONP1 in HEK cells to show the interaction with V5-Flag-tagged WT-GCase

Endogenous coimmunoprecipitation

1 Wash Protein G agarose fast-flow beads in TBS 1X + 0.05% NP40.To this

end, the beads were incubated with lysis buffer on the spinning wheel (25 RPM) for 2 –5 min at 4 °C, followed by a 1 min centrifugation at 500 RPM 4 °C.

2 Repeat step 1 for a total of 3 washes.

3 Incubate 20ul of pure beads with 5ug of anti-LONP1 antibody or 5ug of normal rabbit IgG as control (or 3ug of anti-Hsp60 and 3ug of mouse IgG as control) and add 300ul of washing buffer to each tube.

4 Incubate 2h at 20RPM on a rotating wheel at 4°C

5 Meanwhile, detach HEK cells using Accutase for 5 minutes at 37°C and collect them.

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7 Spin cells in a centrifuge at 250g for 5 minutes at room temperature.

8 Remove the supernatant and wash cells in PBS.

9 Repeat steps 7 and 8 for a total of 2 washes.

10 Lyse cells in 1% TBS + 0.5% NP40 + PI/PHI (Pierce #A32959)

11 After 2h incubation, wash beads again 3 times in washing buffer.

12 Incubate antibody-coated beads with 3.7 ug of lysate on the spinning wheel for 2h.

13 After 2h incubation, wash beads again 3 times in washing buffer.

14 Elute by boiling the beads twice with 2x Laemmli buffer at 95°C for 8min at 300rpm in a thermoblock.

15 Spin beads for 1 minute at 10000 RCF.

16 Collect supernatant and proceed with western blot analysis.