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Coimmunoprecipitation

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We use this protocol and it's working

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Abstract

Co-Immunoprecipitation assays in HEK cells

1 **Transfect HEK293T Cells** - Transfect HEK293T cells with Ezrin/Atg7 cDNA using X-tremeGENE HP (Roche) 36 hours prior to lysis. - Grow cells to 85-90% confluency.

2 **Collect Cells for Lysis** - At 36 hours post-transfection, rinse cells with 1x PBS. - Collect cells for lysis.

3 **Lysis and Protein Extraction** - Briefly vortex cells in chilled membrane solubilization buffer (25 mM HEPES, 150 mM KCl, 1.5 mM MgCl2, 0.5% NP-40, 10% Glycerol) with protease (cOmplete, Roche) and phosphatase (PhosSTOP, Roche) inhibitors.

4 **Equalize Protein Concentrations** - Use the Pierce™ BSA Protein Assay Kit (Thermo Fisher) and CLARIOstar Plus Plate Reader (BMG Labtech) to determine protein concentrations. - Equalize cell lysate concentrations.

5 **Incubate with Magnetic Beads** - Incubate equalized cell lysates with Pierce™ Anti-c-Myc Magnetic Beads (Thermo Fisher) for 6 hours at 4°C while rotating.

6 **Wash Magnetic Beads** - Wash beads with chilled lysis buffer.

7 **Elute Protein Samples** - Elute protein samples from beads by adding 2x Bromophenol Blue-free Laemmli Sample Buffer. - Heat samples to 95°C for 5 minutes.

8 **Perform Western Blot Analysis** - Subject eluted protein samples to western blot analysis using a SimpleWestern Jess (ProteinSimple) automated immunoassay system with a 12-230 kDa Fluorescence Separation module and the manufacturer's protocol.

9 **Primary Antibodies for Detection** - Use the following primary antibodies: - Anti-Myc (Rabbit, 1:40, Cell Signaling Technologies, mAb#2278) - Anti-HA (Rat, 1:20, Roche, 11867423001)

10 **Secondary Antibodies for Signal Detection** - Use the following fluorescently conjugated secondary antibodies at a 1:100 dilution: - IRDye® 680RD Goat anti-Rabbit IgG (LI-COR, 926-68071) - IRDye® 680RD Goat anti-Rat IgG (LI-COR, 926-68076)

11 **Quantify Protein Signals** - Quantify protein signals using the Simple Western Compass software. - Utilize quantitative electropherograms of detected signals. - Background-subtract and normalize Ezrin co-immunoprecipitation signal intensity to Ezrin protein load and Atg7 IP levels for statistical analysis.

12 **Statistical Analysis** - Perform statistical analysis in GraphPad Prism 9. - Use One-way ANOVA with Tukey’s multiple comparisons test. - Set alpha threshold to 0.05.