

Jenny's PERK/IRE1 IP Protocol

Plate cells at a density of $2-2.5 \times 10^6$ per 10cm plate for CHO cells, 10^6 cells per 10cm plate for NIH3T3 cells, or 2×10^6 HeLa cells. After 24h replace media (≥ 2 h) and treat cells w/ UPR drugs.

Wash 10cm plate 2X w/ ice cold PBS + 1mM EDTA.

Collect cells with cell scraper, spin 30s @ 14K rpm, can snap freeze in liquid N₂, or directly lyse cell pellet in 200 μ L ice cold 1% triton buffer. Use 1 plate per IP.

1% Triton buffer	Stock soln	1mL
20mM Hepes	1M pH 7.4	20 μ L
150mM NaCl	5M	30 μ L
10% glycerol	50%	200 μ L
1% triton	10%	100 μ L
1mM EDTA	0.5M pH 8.0	2 μ L
100mM NaF	1M	100 μ L
10mM Na pyrophosphate	100mM	100 μ L
17.5mM b-glycerophosphate	175mM	100 μ L
1mM PMSF	200nM	5 μ L
5mg/mL Aprotinin	5 μ g/mL	1 μ L
2mg/mL Leupeptin	1 μ g/mL	2 μ L
2mg/mL Pepstatin A	1 μ g/mL	2 μ L
mQ H ₂ O		338 μ L

Rotate lysate end-over-end 15min @ 4°C. Spin 10min @ 14K rpm, 4°C to clear.

Transfer cleared lysate to new tube and incubate lysate 1hr w/ 20 μ L of 50% slurry of protein G (Upstate #16-266) in IP buffer @ 4°C rotating end-over-end. Spin down & transfer lysate to clean tube and incubate overnight w/ anti PERK N-18 (SCBT sc-9479), or hIRE1 α (acid). Use 10 μ L PERK Ab, 2 μ L IRE1 (acid) Ab per IP reaction.

Next morning incubate each sample w/ 20 μ L of 50% slurry of protein G in 1% triton buffer @ 4°C rotating end-over-end for 3hr.

Wash beads 3X 5min w/ 1mL ice cold IP buffer, and 1X w/ ice cold PBS +100mM NaF. Remove all buffer with syringe or gel loading tip and resuspend beads in 20 μ L 2X Laemmli w/ 100mM DTT. Heat @ 100°C for 10min, and load onto 7% NuPage Tris-acetate gel. Run @ ≤ 125 V in 1X NuPage TA running buffer (to see alternate forms of PERK or shift of IRE1 you will run ~40-50KD off gel). Transfer gel to nitrocellulose @ 30V, 70min in 1X NuPage transfer buffer w/ 10% MeOH, and **NO SDS**.

For Western: PERK use 1:50 dilution, for IRE1 (acid) use 1:5000. Incubate both o/n @ 4°C in 5% milk.