**Immunoprecipitation (IP)**

Thanh Ngoc Nguyen (Laboratory of Michael Lazarou, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia) (nguyen.tha@wehi.edu.au)

**Buffers and reagents:**

* IP base buffer: 50mM Tris-Cl (pH 7.5 when cold), 150mM NaCl
* Bead equilibration buffer: IP base buffer supplemented with 0.1 % Tween20
* IP wash buffer: IP base buffer supplemented with 0.1 % TX-100 and 1x cOmplete, EDTA-free protease inhibitor cocktail
* Pierce Anti-HA magnetic beads (88836, ThermoFisher)
* Benzonase (E1014-25KU; Sigma)
* Roche cOmplete, EDTA-free protease inhibitor cocktail (4693132001; Sigma)
* Elution buffer: 1x LDS + 0.1 M DTT (diluted from 4x LDS (NP007; ThermoFisher); can be aliquoted and stored at -20 or -80 oC)

**Procedures:**

1. Lyse cell pellets (5-7 mg) in 500 μl IP lysis buffer containing IP base buffer supplemented with 1x cOmplete, EDTA-free protease inhibitor cocktail and 0.1 μl of benzonase and incubate samples on ice for 30 min. Mix the sample by inverting the eppies gently every 5 min.
2. Anti-HA magnetic beads (50 μl of beads slurry for each sample) were washed three times with bead equilibration buffer.
3. Centrifuge the eppies at max speed for 10 min at 4 oC.
4. Carefully transfer cleared lysated into 2 ml eppies and take 50 μl from each tube for “Input” samples.
5. Gently add 1000 μl of IP base buffer containing 1x cOmplete, EDTA-free protease inhibitor cocktail to the rest of each sample to dilute out the detergent.
6. Incubated the diluted cleared lysates with the anti-HA magenetic beads on a rotary mixer for 3 h at 4 oC.
7. Collect beads on a magnetic rack and aspirate the unbounds.
8. Wash with 1 ml IP wash buffer.
9. Repeat steps 7-8 another 4 times. For the last wash, make sure to remove all the liquid off the beads.
10. Elute with 25 μl elution buffer by boiling at shaking at 99 oC for 10 min.