**Staufen-1 RNA Formaldehyde Cross-linking followed by Immunoprecipitation from Tissue Culture Cell**

1. Add cycloheximide to cells (150mm dishes) to 100µg.ml-1 final for 45 minutes.
2. Rinse once with 10 ml PBS + Cycloheximide. Add **1 ml** PBS + Cycloheximide.
3. Scrape off cells using a cell scraper.
4. Collect suspension, pellet cells at 500g for 5 min at RT.
5. Resuspend cells in 30ml of PBS for each plate.
6. Add 81µl of formaldehyde (37%) for a final concentration of 0.1%
7. Incubate at RT for 15 minutes.
8. Add 3.75 ml of 2M glycine; 25mM Tris-HCl pH 7.0
9. Incubate at RT for 5 minutes.
10. Pellet cells at 500g for 5 minutes at 4°C

**Total Lysate, FLAG-IP and Stau1-IP:**

1. Resuspend in ice-cold **1 ml** CLIP lysis buffer for each 150mm plate (i.e. if starting material was 6 plates then lyse in 6ml).
2. Incubate on ice, 10 min.
3. Sonicate the extract at 40% amplitude using a Microtip for 90 seconds in bursts of 15 seconds with 30 second intervals.
4. Spin 15,000 xg, 4°C, 10 min.
5. Transfer 50µl supernatant to a labeled tube to analyze total RNA or protein. Store at -20°C.
6. Transfer remainder of supernatant to tube containing **150 ul** anti-FLAG agarose beads (for each starting 150mm plate), which have already been washed twice with 1 ml IsoWB (Isotonic wash buffer). Nutate at 4°C, 2h.
7. After pelleting the beads, save 50 µl of supernatant to check efficiency of depletion of the target protein. Store at -20°C.
8. Wash beads 2 times with **10 ml** of CLIP lysis buffer (ice-cold).
9. Wash 2 more times in 10 ml of IsoWB (150mM NaCl).
10. After 4th wash, transfer beads to a new 15ml conical tube.
11. Wash beads 4 more times in 10 ml of IsoWB.
12. Add **125 ul** of IsoWB+250µg.ml-1 of Flag-peptide (for each 150mm plate).
13. Elute by gentle shaking at 4°C for 2 hrs (vortex setting 2-3).
14. Recover the supernatant and transfer to a new eppendorf tube. To the beads, add **400 ul** or **800 ul** of IsoWB. Mix to resuspend beads and spin down for 1min at 1000 rpm.
15. Pool **355 ul** or **860 ul** supernatant with elution in previous step.
16. Incubate supernatant with anti-Staufen-1 antibody (15µl of Abcam 105398 + 10 of MBL antibody) conjugated to ProteinG-Dyna-beads, which have already been washed twice with 1 ml of PBS + 0.02% Tween20 and twice with 1 ml of IsoWB.
17. Nutate at 4°C, 2h.
18. Wash beads 6 times with 1 ml IsoWB (ice-cold). For each wash, capture dyna-beads and remove the buffer. Add fresh buffer and completely resuspend the beads.
19. After last wash, add 500 µl of Clear Sample buffer. Incubate at RT for 5 min, and then heat at 75°C for 45min under agitation (500rpm).
20. Capture the beads on the magnet and transfer all the supernatant (elution) to a new tube. Save 20µl for analysis of proteins on Western blot and proceed to RNA extraction from the remaining.

**Antibody-Dynabead Conjugates**

1. For each IP from a 15-cm plate, wash 35ul of ProteinG Dynabead conjugates three times with 1ml of PBS = 0.02% Tween20.
2. Resuspend beads in 800ul of PBS + 0.02% Tween20 and add antibodies. *When preparing conjugates for multiple IPs with same antibody, assemble one conjugation reaction with volume not exceeding 800ul. The conjugation is more efficient when the mix is more concentrated.*
3. Nutate at RT for 1-2 hours.
4. Capture the beads on magnet and remove the supernatant.
5. Wash beads 2 times with PBS + 0.02% Tween20 and 2 times with ice-cold IsoWB. Keep the beads on ice in at least 100ul of IsoWB if not needed for IP right-away. Remove all buffer before adding cell extract for IP.

**Hypotonic Lysis Buffer**

20mM Tris-HCl pH7.5

15mM NaCl

10mM EDTA

0.5% NP-40

0.1% Triton-X-100

Protease inhibitor cocktail (Roche)

\* *add fresh every time*

**CLIP lysis buffer**

1X PBS

0.1% SDS

0.5% Sodium Deoxycholate

0.5% NP-40

**IsoWB**

20mM Tris-HCl pH7.5

150mM NaCl

0.5% NP-40

**Clear Sample Buffer**

100mM Tris-HCl pH6.8

4% SDS

10mM EDTA

100mM DTT\*

\* *add fresh every time*