**Staufen-1 RNA Immunoprecipitation from Tissue Culture Cell**

1. Grow Flp-In FLAG-Stau1 cells in a **100mm** or **150mm** plates, rinse once with PBS. Add **5 ml** or **10 ml** PBS.
2. Scrape off cells using a cell scraper.

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

1. Collect suspension, pellet cells at 500g for 5 min at 4°C.
2. Resuspend in ice-cold **1 ml** or **3 ml** hypotonic lysis buffer [Use 15 ml conical tubes for 3 ml size].
3. Incubate on ice, 10 min.
4. Sonicate the extract at 40% amplitude using a Microtip for a total time of 30 seconds in bursts of 2 seconds with at least 10 second intervals (If you want to prepare libraries for sonication only experiments you need to sonicate at 40% amplitude for a total time of 90 seconds using 5 second burst and 30 second intervals between bursts).
5. Add NaCl to 300 mM.
6. Incubate on ice 5 min.
7. Spin 15,000 xg, 4C, 10 min. If you skipped step 7, the resulting supernatant is primarily a cytoplasmic extract.
8. Transfer 50µl supernatant to a labeled tube to analyze total RNA or protein. Store at -20C.
9. Transfer remainder of supernatant to tube containing **80 ul** or **150 ul** anti-FLAG agarose beads, which have already been washed twice with 1 ml IsoWB (Isotonic wash buffer). Nutate at 4C, 2h.
10. After pelleting the beads, save 50 µl of supernatant to check efficiency of depletion of the target protein. Store at -20C.
11. Wash beads 4 times with **1 ml** or **3 ml** IsoWB (ice-cold).
12. After 4th wash, transfer beads to 1.5ml eppendorf (resuspend beads in 1ml IsoWB and pipet into eppi).
13. Add **40 ul** or **112 ul** of IsoWB+1U/ul RNase I. Incubate with intermittent shaking at 37C for 10 min (If you are doing the sonication only experiment do not perform this RNase Step and skip directly to step 17).
14. Wash beads 4 times with **1 ml** or **3 ml** IsoWB (ice-cold) (Transfer beads back to a falcon tube if doing 3ml washes).
15. Remove all wash buffer and add **40 ul** or **88 ul** of IsoWB + 250ug/ml FLAG peptide.
16. Elute by gentle shaking at 4C for 2 hrs.
17. Remove **45 ul** or **80 ul** of elution. To the beads, add **400 ul** or **800 ul** of Isotonic lysis buffer supplemented with 1 mg/ml BSA (NEB). Mix to resuspend beads and spin down for 1min at 1000 rpm.
18. Pool **355 ul** or **860 ul** supernatant with elution in previous step.
19. Incubate supernatant with anti-Staufen-1 antibody (15µl of Abcam 105398 + 10 of MBL antibody) conjugated to ProteinG-Dyna-beads (see protocol below), which have already been washed twice with 1 ml IsoWB (Isotonic wash buffer).
20. Nutate at 4C, 2h.
21. 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.
22. After last wash, add 200 µl of Clear Sample buffer. Incubate at RT for 5 min, and then heat at 95˚C for 2min. Keep mixing the tubes occasionally.
23. Capture the beads on the magnet and transfer all the supernatant (elution) to a new tube. Save 2-4µl 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 Cit-PO4 buffer (below).
2. Resuspend beads in 800ul of the Cit-PO4 buffer and add 11µl of anti-Staufen 1 (Abcam 105398) and 7.5 µl of Anti-Staufen 1 (MBL) antibody per IP. *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 the Cit-PO4 buffer 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

10ug/ml Aprotinin\*

1ug/ml Leupeptin\*

1uM Pepstatin\*

1mM PMSF\*

40U/ml RNaseIn\* (I do not use this anymore)

\* *add fresh every time*

**Isotonic lysis buffer**

Hypotonic Lysis buffer with NaCl at 150mM

**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*

**PrG Wash Buffer (Citrate-PO4 Buffer, pH 5.0)**

 4.7g Citric Acid (MW=192)

 9.2g Na2HPO4 (Dibasic) dehydrate (MW=178)

 Water to 1L. Store at RT