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Pull-down Assay of Biotin-labeled Histone peptides

Binding Buffer
50 mM Tris pH 7.5
150-300 mM NaCl
0.05-0.1% NP-40

1. Add 1 µg different biotinylated histone peptides (1mg/ml, Upstate Biotech.) into 200-300µl of binding buffer with 1 µg of each GST, GST-PHD and GST-BD1 fusion proteins; also incubate fusion protein without histone peptide as negative control. Rotate at 4°C for 4 h to O/N.
   Note: Spin at 13K for 10 min before take sample of GST fusions purified by Glutathione sepharose beads.
2. Prepare the Streptavidin Sepharose beads (Amersham). Use 15 µl Streptavidin beads (~20 µl of 75% slurry) for each binding assay. For 30 assays, using a cut tip P1000 to take about 0.67 ml of the (75%) slurry and transfer to a 15 ml Falcon tube, bring the volume to 5 ml -10 ml with cold lysis buffer, spin the beads at 500g for 3 min (2K rpm of JA 5.3 rotor for 2 min). Remove the supernatant and wash the beads at least two more times with lysis buffer. After the final wash, bring the volume to 1 ml and resuspend the beads (to 50% slurry).
3. Add 20-30 µl of 50% slurry into each tube. Rotate at 4˚C for 1 hr.
4. Spin at 2-4K for 1-2 min at table-top centrifuge. Save the supernatant as Sample FT (Flowthrough).
5. Wash the beads with 1 -1.5 ml of Binding Buffer for 3x times.
6. Resuspend beads in 60 µl of 2x SDS sample buffer. Boil it and ready for SDS/PAGE. For 10% input, take 0.1 µg protein into 60 µl SDS sample buffer. Load 10 µl for each Western.