Pull-down Assay of Biotin-labeled Histone peptides

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

1. Thaw GST-tagged proteins on ice. Spin at 13K for 10 min, and carefully take proteins from the top of the solution. **Avoid taking any Glutathione sepharose beads with your samples!**
2. Dilute proteins for binding: each binding requires 1-2 μg of GST-tagged protein in 300μl of binding buffer. For 8 binding assays, make 3 ml of binding buffer contains 10-20 ug GST-protein which includes a tube (300ul) of fusion protein without histone peptide as negative control. Also save a tube as input.
3. Add 1 μg different biotinylated histone peptides (1mg/ml) into each tube. (not the input tubes)
4. Rotate at 4ºC for 4 h to O/N.
5. Prepare the Streptavidin Sepharose beads (Amersham). Use 12-15 μl Streptavidin beads for each binding assay. Invert the bottle gently to mix the beads well before taking beads. For 30 assays, using a cut tip P1000 to take about 0.4-0.45 ml of the slurry and transfer to a 15 ml Falcon tube, bring the volume to 5 ml -10 ml with cold binding 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 binding buffer. After the final wash, bring the volume to 0.9 ml and resuspend the beads.
6. Add 30 μl of 50% slurry into each tube. Rotate at 4˚C for 1 hr.
7. Spin at 2-4K for 1-2 min at table-top centrifuge. Save the supernatant as Sample FT (Flowthrough) if needed.
8. Wash the beads with 1 ml of Binding Buffer for x times. Rotate at 4oC for 5 min for each wash.
9. Resuspend beads in 60 μl of 2x SDS sample buffer. Boil it and ready for SDS/PAGE. For 10% input, take 30 ul of samples from the saved input tube, add 30 ul 2x SDS sample buffer and boil. Load 10 μl for each Western.