Appendix

Peptide Immunoprecipitation with anti-diglycine lysine Antibody Beaded Agarose

1. Wash 20~40 µl of drained anti-diglycine lysine antibody beaded agarose with 0.5 ml of ice-cold PBS, three times wash;
2. Dissolve the dry tryptic peptides with appropriate volume of IP Buffer (the total volume should be not less than 200 µl); The ratio of peptides to beads is 3~6 mg peptides to 20~40 µl of drained antibody beads;
3. Clear the solution by centrifugation 12,000 x g for 10 min at 4°C;
4. Transfer the supernatant into 0.6 ml eppendorf tube containing pre-washed antibody conjugated beads. Seal the tube with parafilm to avoid leakage;
5. Incubate overnight with gentle end-to-end rotation at 4°C;
6. Centrifuge 500 x g for 30 s to pellet the antibody conjugated beads;
7. Add 0.5 ml of Wash Buffer I to the beads and wash the beads by inverting tube 15 times; Repeat wash three times;
8. Add 0.5 ml of Wash Buffer II to the beads and wash the beads once by inverting tube 15 times;
9. Add 0.5 ml of Milli-Q or equivalently purified water to the beads and wash the beads by inverting tube 15 times; Repeat wash twice;
10. Centrifuge at 500 x g for 30 s to pellet the beads;
11. Elute the bound peptides with 100 µl of Elution Buffer for 1 min with gentle end-to-end rotation at room temperature; Three times elution;
12. Combine the elutes and centrifuge 1,000 x g for 1 min to save the elutes;
13. Dry the elutes for the following mass spectrometry analysis.