1. Dissolve lyophilized peptides, from tryptic digestion of purified ubiquitinated proteins, in 200 µL of 150 mM NaCl, 50 mM Tris-HCl (pH 7.4) and 2 mM EDTA. Make sure that the pH of the solution is ~7.4.

2. Incubate the sample in boiling water for 10 min to inactivate residual trypsin. Cool sample to 4 °C on ice.

3. Incubate the peptide sample with 20 µL of Affi-gel 10 resin coupled with anti-diglycyl-lysine antibody for 4~8 h at 4 °C on an end-over-end rotator. Alternatively, the incubation step can be extended to overnight at 4 °C.

4. Mix the beads with the liquid in the 0.65 mL tube using a pipette tip and transfer beads to a pierce micro-spin column using a large orifice pipette tip. The micro-spin column used here is from Pierce (Thermal Scientific, the last item in this webpage, http://www.piercenet.com/Products/Browse.cfm?fldID=863EB77C-4B2C-4946-B933-99A9165D5684&WT.mc_id=keyname). Note: Carefully remove any bubbles using gel loading tip if necessary.


6. Use 1 mL syringe to slowly push the liquid through the micro-spin column and collect the flow-through to the same 0.65 mL Eppendorf tube. Make sure to keep a small amount of liquid above the resin in the spin column and do not let the resin dry.

7. Mix the flow-through with the residual beads in the 0.65 mL Eppendorf tube and reload the flow-through into the micro-spin column twice using a 200 µL pipette tip and repeat step 6. Note: Remove the syringe before reloading the sample to avoid introducing bubbles. Carefully remove bubbles using gel loading tip if necessary. It is not necessary to remove the Luer-lok adaptor.

8. Wash beads twice with 0.5 mL 2XPBS and twice with 0.5 mL 1XPBS using the same procedure as step 7. Note: Do not let the resin dry.

9. Elute ubiquitin signature peptides six times with 1X bed volume of 10 mM sodium citrate solution (pH 3, The pH is adjusted by HCl and the lowest pH tested is pH 2.5). Alternatively, 0.1 % TFA can be used to elute the peptides, which can improve the yield.
of ubiquitin signature peptides up to 20%. Note: Keep the beads in the elution solution for 2 ~ 5 min before eluting by slowly pushing the 1 mL syringe.

10. Reduce the volume of the eluate to ~ 20 µL with vacuum centrifuge at 25 °C.

11. Adjust volume with 0.1% TFA to 20 µL. Samples can be stored overnight at -20 °C.

12. Perform MS analysis.

13. If sodium citrate is used to elute the peptides, wash resin with 500 µL of 100 mM sodium citrate (pH 3) twice in 2 min and equilibrate the beads with 500 µL PBS twice and store in PBS with 0.02 % NaN₃ in 4 °C. The antibody activity will be slightly reduced after each use. If 0.1 % TFA is used to elute the peptides, discard the column and antibody resin.