Sepharose 4B highly efficient enrichment protocol

1. To remove protecting groups (lactose) 1.1 g of dried Sepharose 4B powder was weighed and washed four times for 15 minutes on each occasion in 50 ml of 1mM HCl at ambient temperature, followed by washing with the same volumes of MilliQ water and coupling buffer (0.2 M NaCl and 0.1 M $NaHCO_3$) Note, that the decreasing of ion strength (i.e NaCl concentration) may increase unspecific binding and primary aminogroups containing compounds.

2. The prepared 20% Sepharose 4B gel suspension in coupling buffer was then immediately transferred to biological sample with a 1:10 (v/v) ratio and incubated for 14 hours at $4^{o}C$ with end-over-end gentle stirring. Estimate that 1mg of BrCN-activated Sepharose 4B with particle size 40-120 um enables enrichment of about 35 mg of protein, thus try to calculate the final amount of Sepharose 4B with 50-100 excess of binding capacity.

3. After binding reaction completed, the suspension of beads was then left to sediment under gravity and 0.2 M glycine solution in 0.2 M NaCl and 0.1 M $NaHCO_3$ was added for free surface blocking for 2 hours at ambient temperature. The blocking solution was decanted and the beads washed twice from an excess of glycine with the same volume buffer as that used during protein coupling, and with 1/10 of this volume of trypsin digestion buffer (37 mM Triethylamminia hydrogen carbonate, 6.7 % acetonitrile amd 4 mM $CaCl_2$ pH 8.3).

4. 1500 ng trypsin (Trypsin porcine modified sequencing grade, Promega) for each 150 ug of proteins were added, and the reactions were incubated at $43^{o}C$ for 6 hours. Then 750 ng of trypsin for each 150 ug was added again and the reaction was incubated overnight at $37^{o}C$ with gentle end-over-end stirring.

5. After the hydrolytic reaction was complete, the bead suspensions were left to form a sediment under gravity. The supernatant was collected and the Sepharose 4B beads were washed twice in 1/100 of the original volume in 37 mM TEABC pH 8.3. The eluted fractions were collected and combined.

6. The resulting fractions were dried at $30^{o}C$ and dissolved in 7 µl of 5% FA. The peptide mixtures were then transferred into deactivated glass capillaries for LC-MS/MS analysis.