His-Protein and IVT Binding Assay

1) Use 15 ug his-proteinX (test) and 15 ug of his-proteinY (neg. control) or no protein (neg. control)
2) Wash 14 uL of 50% Ni++-NTA Qiagen beads in 400uL TBST, repeat (in 0.5mL tubes)
3) Add 50 uL of TBST (supplemented with 10mM B-Me) + His-tagged proteins of interest
4) Mix for 1 h in vibrating mixer (level 3-4) at RT (do binding in cold if protein is unstable at RT)
5) Remove supernatant
6) Add 50 uL of TBST + 5% milk + 10mM B-Me
7) Mix for 1 h in vibrating mixer (level 3-4) at RT
8) Remove supernatant (beads are hard to see, so be careful)
9) Add 50 uL of TBST + 5% milk + 10mM B-Me
10) Add 5 uL in vitro translated protein of interest. Keep 1 uL as input.
11) Mix for 1 h in vibrating mixer (level 3-4) at RT
12) Wash beads (red) 4X with 400uL TBST + 10mM B-Me
13) Remove all extra sup with pipette and add 20ul of 2X SDS-SB and boil 5-10 min
14) Also add 20ul 1X SDS-SB to input
15) Load 10 uL of input (10%), and 18 uL of bound
16) Run through stacker at 80 V and then 100 V until dye front reaches bottom of gel
17) Carefully remove gel and cut off stacking gel
18) Stain gel in coomassie blue stain at RT on rocker for 20 min or until gel is blue
19) Remove staining solution (reuse) and wash gel in dH_20 once
20) Destain gel in destaining solution with Kimwipes on rocker at RT for ~20 min or until bands are visible
21) Rinse gel in dH_20
22) Dry gel on dH_20 soaked filter paper for 1 hr in gel dryer
23) Tape dried gel/filter paper down in phosphoimager cassette
24) Mark molecular weight ladder with excess ^{35}S-IVT reaction mixture
25) Place phosphoimager screen over gel and close cassette
26) Expose overnight
27) Image next day on Fuji PhophoImager