Invitro Binding Assay Protocol

Binding Buffer

1X Phosphate Bufferred Saline (PBS)
0.1% NP40
0.5mM DTT
10% Glycerol
1mM PMSF
2 μg/ml Aprotinin

Preclear

To 500 μl of binding buffer add 20 μl of Glutathione Sepharose beads and 40 μl of ^{35}S labled IVT- protein and rotate at 4^0C for 45 minutes. Centrifuge for 1 min at 15,000rpm at 4^0C and discard the pellet.

GST Control

To the supernatant add GST control beads prepared from the pGEX vector (Use same amount of GST protein as that of the experimental Protein from Coomassie staining the gel) and again rotate at 4^0C for 30 min. Centrifuge as before and save both the pellet and the supernatant. The pellet should be washed with the binding buffer 5 times and should be used as GST control. After washing the beads well with out vortexing add SDS-lysis buffer.

GST-Protein

To the supernatant add GST-protein beads and rotate for 2 hours at 4^0C. Centrifuge at 4^0C as before and discard the supernatant. Wash the beads 5 times with binding buffer and use it as GST-Protein. Add SDS-lysis buffer to the GST-protein beads.

Run it on the right percentage of Gel and if needed use IVT- luciferase control and treat it the same way as that of IVT protein.