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NBP1-30127 Protocol

Immunoprecipitation (IP) protocol

1. Dilute sample (200 - 500 ug of total protein) with Correction Buffer (2.5% v/v Nonidet P-40, 5% w/v sodium deoxycholate, 0.5% w/v SDS in ddH2O) in ratio 4:1 (v/v).
2. Add 5 ul of the antibody, mix gently and incubate for 1 hour on ice.

3. Mix with 50 ul of ProteinG-Sepharose (washed with 10mM Tris-HCl, pH7.5*) and incubate for 30 minutes at 4 degrees C with gentle shaking.

a. *NOTE: Washing of ProteinG-Sepharose with 10mM Tris-HCl, pH7.5: Resuspend 50 ul of ProteinG-Sepharose in 1 ml of 10mM Tris-HCl, pH7.5 by precise inverting of the tube several times. Centrifuge for 1 min, 900xg at 4 degrees C and discard supernatant. Avoid of wasting ProteinG-Sepharose agarose gel beads during discarding. Repeat this procedure for 3 times.

4. Centrifuge ProteinG-Sepharose immunocomplex for 2 min, 900xg at 4 degrees C and discard supernatant. (DO NOT spin any faster as this can cause the binding affinity to decrease).
5. Wash the pellet 3 times with 1 ml of RIPA Buffer (10mM TRIS-HCl, pH7.5, 140mM NaCl, 1% v/v Nonidet P-40, 1% w/v sodium deoxycholate, 0.5% w/v SDS v ddH2O).
a. IMPORTANT: Avoid of wasting/discarding ProteinG-Sepharose immunocomplex.
6. Wash sediment with 1 ml of 10mM TRIS-HCl, pH7.5, centrifuge sample (agarose gel beads) for 1 min, 900xg at 4 degrees C and discard the supernatant.
7. Dissociate immunocomplex from ProteinG-Sepharose with the help of Reduction Buffer (125mM TRIS- HCl, pH6.8, 3.3% SDS, 5% beta-mercaptoethanol). Mix the sample with 30 ul of Reduction Buffer, shake gently and incubate for 5 minutes at 65 degrees C.
8. Centrifuge at 3000xg for 5 minutes and transfer the supernatant (immunoprecipitated proteins) to new tube.

9. Separate the immunoprecipitated protein by 1D SDS-PAGE