Immunoprecipitations

Protocol:

1. Use 300-400 μg protein per IP
2. Add primary antibody as directed (usually ~2 μg for a monoclonal antibody)
3. Incubate 1 to 2 hr on ice
4. For polyclonals: Add 25 μl Pansorbin (10% suspension), washed and preincubated in RIPA buffer containing 10 mg/ml BSA
5. For monoclonals: Wash and preincubate Pansorbin (10% suspension) in RIPA buffer containing 10 mg/ml BSA. Add 1 μl rabbit anti-mouse IgG/25 μl Pansorbin (10% suspension) and incubate on ice for 45 min. Spin down Pansorbin and remove supernatnat. Wash once in IP buffer containing 10 mg/ml BSA. Make up to original suspension volume in RIPA buffer and add to IP's.
6. Incubate IP's with Pansorbin for 30 min on ice.
7. Spin down in a microfuge for 10 to 15 sec and aspirate the supernatant
8. Wash pellet three times by resuspending in RIPA buffer, pelleting, and removing the supernatant.
9. Resuspend final pellet in 50 μl RIPA buffer and add 50 μl SDS sample buffer.
10. Boil 3 min, pellet the Pansorbin by centrifugation in a microfuge for 30 sec
11. Load the supernatant onto an SDS gel

Note:

You may substitute anti-mouse IgG-agarose or protein G-agarose for the Pansorbin plus rabbit-anti-mouse antibodies for use with monoclonal primary antibodies. Use according to manufacturer's instructions.