

NB200-174 Protocol**Immunoprecipitation Protocol (NB200-174)**

Immunoprecipitation

1. Total cell lysates (0.25 mg) from NIH3T3 cells which have deleted the Arf gene and from NIH3T3 cells expressing an HA-tagged version of p19Arf were incubated with control rat IgG or anti-p19Arf (NB 200-174) and protein G-Sepharose.
2. Precipitated proteins were resolved by SDS-PAGE and analysed by Western blotting using an anti-HA antibody. Unprecipitated total cell lysates (25 ug, equivalent to 10% of immunoprecipitation) from Arf null NIH3T3 cells and HA- p19Arf expressing cells were also analysed by Western blotting, as input controls.