

NB500-211 Protocol

Immunoprecipitation protocol specific for CAF-1 p60 Antibody (NB500-211)

CHAF1B Antibody (SS 24 1-68): https://www.novusbio.com/products/chaf1b-antibody-ss-24-1-68_nb500-211
Procedure Guide for Monoclonal anti- CAF-1 p60 (clone 24)
Catalog# NB 500-211

Immunoprecipitation Procedure

- 1) Cells were prepared in 0.4-1% NP-40 lysis buffer.
- 2) After osmotic shock and nuclear extractions of the cells, use the following buffer: 25mM Tris, 150mM NaCl, 1mM EDTA, 1mM DTT, and 0.01% NP-40.
- 3) Clarify the extracts with two successive 5 minute clearing spins (in a microfuge).
- 4) Preclear the extracts with some protein G beads (~20 ul).
- 5) Incubate the extracts with ~ 0.1-1ug of anti-CAF-1p60-24 [NB 500-211]/ control antibody for 1-3 hours.
- 6) Spin the antibody/extract for 5 minutes.
- 7) Incubate the antibody/extract with protein G beads, rotating for 1-2 hours.
- 8) Wash the beads, twice, for 5 minutes, at low ionic strength [150mM NaCl] in 1ml volume (*Optional: wash again, twice, at high ionic strength [400mM NaCl]).
- 9) Resuspend the washed beads in Laemlli sample buffer and boil for 5 minutes.

NOTE:

- a. All steps for IP are performed at 4C.
- b. CAF-1p60 is phosphorylated and contains a PEST sequence. Therefore, it is recommended that protease and phosphatase inhibitors be added to all buffers.
- c. The protocol for crosslinking CAF-1p60 to protein G beads was obtained from the Harlow and Lane book, Using Antibodies.