Nucleosome Immunoprecipitation

Revised by S Houston
August 2007

Procedure

1. Obtain mononucleosomes in MNase digestion buffer (e.g. as described in MNase Digestion for Native ChIPs).
2. Determine mononucleosome concentration by UV spectroscopy.
3. Add sufficient MNase digestion buffer to dilute100 µg of nucleosomes to a volume of 750 microliters.
4. Obtain ammonium sulfate precipitated IgG fraction of antibody (e.g. as described in Ammonium Sulfate IgG Precipitation) and resuspend in the same amount of PBS as the original serum.
5. Add IgG solution to dilute mononucleosomes.
   Incubate for 2 hours, rotating, at 4°C.
6. Precipitate complexes using Protein A conjugated Sepharose beads (Amersham Biosciences), with 0.75 microliters of beads per microliter of Antibody.
   Incubate for 30 minutes rotating at 4°C.
   6.1. Mononucleosomes will begin to bind non-specifically to the beads as incubation time increases.
7. Wash beads sequentially in 1mL PBS containing 50, 100 and 150 mM NaCl with rough and thorough pipetting.
8. Elute the bound material in TE buffer with 1% SDS by intermittent vortexing for 30 min.