

NB100-744 Protocol

Protocol specific for Cytosine (5-Methyl) Antibody (NB100-744)

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Use 0.5 to 1 ug of sonicated or enzyme-digested genomic DNA

IP:

- 1) Dilute to a final volume of 100 ul and a final concentration of 0.15% SDS, 1% triton x-100, 150mM NaCl, 1mM EDTA pH8.0, 0.5 mM EGTA pH8.0, 10 mM Tris pH8.0, 0.1 % BSA, 7 mM NaOH (crucial), anti-5mC (up to 30 ug of antibody for saturating conditions), Prot A/G beads.
- 2) Rotate o/n at 4C.

Wash:

- 1) 2x in: 0.1% SDS, 0.1% sodium deoxycholate, 1% triton, 150 mM NaCl, 1mM EDTA pH8.0, 0.5 mM, EGTA pH8.0, 10 mM Tris pH8.0
- 2) 1x in: same as above, but NaCl is 500mM
- 3) 1x in: 0.25 M LiCl, 0.5% sodium deoxycholate, 0.5% NP-40, 1mM EDTA pH8.0, 0.5 mM, EGTA pH8.0, 10 mM Tris pH8.0.
- 4) 2x in: 1mM EDTA pH8.0, 0.5 mM EGTA pH8.0, 10 mM Tris pH8.0.

Elute:

- 1) Elute in 1% SDS, 100mM NaHCO₃.
- 2) Purify DNA using phenol/chloroform extraction and EtOH precipitation.
- 3) Analyze using real-time PCR.