05/19/2016 Immunoprecipitation test for polβ *antibody* (*Novus Biologicals*, $NB100-91734$)

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A. Materials used:

1. DNA Polymerase beta antibody (NB100-91734) purchased from Novus Biologicals, USA.
2. **10X Cell Lysis Buffer** from Cell Signaling Technology (CAT#9803): To prepare 10 ml of 1X cell lysis buffer, add 1 ml 10X cell lysis buffer to 9 ml Milli Q water, mix. Add 1 mM PMSF and proteinase inhibitor cocktail immediately prior to use.
3. **Protein A Agarose Beads from Cell Signaling Technology (CAT# 9863)**.
4. **Normal rabbit IgG from Santa Cruz Biotechnology (CAT# sc-2027).**
5. **Human U2OS cells (ATCC# HTB-96) cultured in DMEM + 10% FBS + pen/Strep.**
6. **Purified recombinant human DNA polymerase β (His-tagged) were prepared by the Demple Lab, Department of Pharmacological Sciences, Stony Brook University, Stony Brook, New York).**

B. Preparing Cell Lysates

1. To harvest cells, remove media and rinse cells once with ice-cold 1X PBS.
2. Remove PBS and add 0.4 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate on ice for 5 min.
3. Scrape cells off the plate and transfer to microcentrifuge tubes. Gently rock the suspension on an orbital shaker at 4°C for 15 minutes to lyse cells.
4. Microcentrifuge for 15 min at 4°C, 14,000 x g and transfer the supernatant to a new tube. The supernatant is the cell lysate.

C. Immunoprecipitation

1. Protein A agarose beads were washed by PBS and restored to 50% slurry with PBS.

2. To pre-clear cell lysates, 20 µl of 50% protein A bead slurry were added to 200 µl cell lysate at 0.6 mg/ml.

3. Incubate with rotation at 4°C for 30 min.

4. Microcentrifuge for 10 min at 4°C. Transfer the supernatant to a fresh tube.

5. DNA polymeraseβ antibody (Novus biologicals, NB100-91734) was added to 200 µl of pre-cleared cell lysate at 0.6 mg/ml to final antibody concentration of 2µg/ml.

7. Incubate for 2 hours on a rotating wheel at 4°C.
8. Add 20ul of protein A beads (50% bead slurry), incubate with gentle rotating for 2 hr at 4°C.
9. Microcentrifuge for 30 sec at 4°C. Save supernatants for control loading and wash pellet five times with 500 µl of 1X cell lysis buffer. Keep on ice between washes.

D. Sample Analysis by Western Immunoblotting

1. Resuspended the pellet in 20 µl of 2X SDS sample buffer.
2. Samples were boiled for 5 min and microcentrifuge for 1 min at 14,000 x g.
3. Load samples (including supernatant and input samples) onto a 12% gel for SDS-PAGE.
4. Analyze sample by western blot.

E. Result:

It turned out that this antibody is not suitable for IP application, as shown on the image:

1) NB100-91734 antibody didn't pulldown endogenous DNA polymerase β from cell lysates (lane 7), which is the same as the normal rabbit IgG control (lane 6). It is believed that DNA polymerase β protein stayed in the supernatant fraction, although lane 2 and lane 3 didn't show the polymerase β band, that is due to the fact that 10% of supernatant just equal to 12ug of cell lysate, which is below the detectable level in view of the fact that only 30ug of cell lysate could show a specific endogenous polymerase β band (lane 10).
2) NB100-91734 antibody did pulled down a portion of purified recombinant human polymerase β (His-tagged) (lane 8 and 9), but its binding capacity is very limited, seeing that 10% supernatant already showed pretty strong bands in both 100ng and 25ng IP reactions (lane 4 and 5).

   Conclusion:

   CAT# NB100-91734, DNA polymerase β antibody is not suitable for immunoprecipitation use.

05/19/2016 DNA polβ antibody (NB100-91734) IP testing