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NB100-317 Protocol

Immunoprecipitation protocol for Telomerase reverse transcriptase Antibody (NB100-317)

TERT Antibody (2C4): https://www.novusbio.com/products/tert-antibody-2c4_nb100-317
Immobilization of Anti-hTERT anibody
All reagents were from the Seize Primary Mammalian IP Kit.
50 ml of mouse ascites (3.3 mg/ml) was diluted with 350 ml of coupling buffer and coupled to 400 ml of AminoLink Plus slurry per the manufactures instructions. Greater than 80% of the protein in the antibody solution were coupled to the beads.

Immunoprecipitation

1. hTERT was synthesized in rabbit reticulocytes using a pET vector and [35S]-methionine was used to allow visualization of the protein.
2. Beads were washed 2X with wash buffer (WB1): 20 mM Tris-acetate, pH 7.5, 10% glycerol, 1 mM EDTA, 5 mM MgCl2, 100 mM potassium glutamate, 0.1% IGEPAL, and 1 mM DTT, then blocked twice with 250 mL of blocking buffer (20 mM Tris-acetate, pH 7.5, 10% glycerol, 1 mM EDTA, 5 mM MgCl2, 100 mM potassium glutamate, 0.1% IGEPAL, 1 mM DTT, 0.5 mg/mL lysozyme, 0.5 mg/mL BSA, 0.05 mg/mL glycogen, and 0.1 mg/mL yeast RNA) for 15 min at 4C.
3. In between each washing and blocking step the beads were precipitated by centrifugation at 1500g and the supernatant was removed.
4. 50 mL of blocking buffer was then mixed with the 50 mL RNA/protein sample and centrifuged at 17 000g for 10 min at 4C to remove any precipitates.
5. This supernatant was then added to the blocked beads and the samples were mixed on a rotary platform for 2 h at 4C.
6. Following mixing, the beads were washed three times with 325 mL of Wash Buffer #2 (20 mM Tris-acetate, pH 7.5, 10% glycerol, 1 mM EDTA, 5 mM MgCl2, 300 mM potassium glutamate, 0.1% IGEPAL, and 1 mM DTT) and twice with 325 mL of TMG (10 mM Tris-acetate, pH 7.5, 1 mM MgCl2, and 10% glycerol).
7. The beads were precipitated by centrifugation at 1500g in between each wash and the supernatant was removed.
8. The beads were then resuspended in 1X SDS gel loading buffer containing 10 mM DTT and analyzed by SDS PAGE.
9. The immunoprecipitation was also performed on 1x10(7) A549 cells.
10. The beads were assayed by TRAP assay.

Results: IP of [35S]-labled hTERT resulted in 10% yield. This is the same efficiency we observed for anti-HA beads used to IP HA tagged hTERT. IP of telomerase from cells allowed isolation of beads that contained telomerase activity.

Conclusion: We successfully immobilized anti-hTERT antibodies on AminoLink beads using the Seize kit from Pierce. These can be used to immunopurify telomerase. The efficiency should be optimized, but the preliminary results are promising.

Protocol courtesy of Pamela K. Dominick and Michael B. Jarstfer from University of North Carolina, Chapel Hill.