Immunoprecipitation with Topo I Monoclonal Antibody

1. Prepare nuclear protein from HeLa cells (800ug ~ 1mg).

2. Dilute the nuclear protein to approximately 1mg/ml total cell protein with PBS to reduce salt concentration in the buffer.

3. Add the 2ug of the Topo I monoclonal antibody to 1ml of nuclear extract.

4. Gently mix the nuclear extract/antibody mixture for either 2 hours or overnight at 4C on a rocker.

5. Capture the immunocomplex by adding 50ul of protein G sepharose bead slurry (50%) and gently rock for 2 hours.

6. Collect the sepharose beads by pulse centrifugation (5 seconds in the microcentrifuge at 14,000 rpm). Discard the supernatant fraction and wash the beads 3 times with 1ml of ice-cold PBS.

7. Resuspend the beads in 30ul of 2 X SDS loading buffer and mix gently.

8. The sepharose beads are boiled for 5 minutes to dissociate the immunocomplexes from the beads. The beads are collected by centrifugation and SDS-PAGE is performed with the superna- tant fraction.