

Small Scale Preparation of Topo I and II Extracts from tissue culture cells (optimized for HeLa cells)

Perform all operations using an ice bucket. These enzymes inactivate readily and are easily proteolyzed.

1. From 1-2 100 mm petri dishes, scrape up cells into medium.
2. Pellet cells 800 xg for 3 min in the cold.
3. Resuspend Cell pellet in 3-5 ml of ice cold TEMP buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 4 mM MgCl₂, 0.5 mM PMSF) and disperse clumps by pipetting up and down.
4. Repeat centrifugation step and resuspend in 3 ml of TEMP and disperse as above.
5. Leave on ice 10 min.
6. Dounce in tight fitting homogenizer 6-8 strokes (check for nuclei by phase microscopy).
7. Pellet nuclei by centrifugation at 1500 xg for 10 min. (cold).
8. Resuspend the nuclear pellet in 1 ml of cold TEMP. Optional: Transfer to an eppendorf microfuge tube. Repeat step 7 spin or pellet in Microfuge (at 4°C) for 2 min.
9. Resuspend nuclear pellet in a small volume (no more than 4 pellet volumes) of TEP (same as TEMP but lacking MgCl₂).
10. Add an equal volume of 1M NaCl, vortex, leave on ice for 30-60 min.
11. Spin in ultracentrifuge at 100,000 xg for 1 hr. (cold). Alternatively, you may be able to spin in the cold in a microfuge at 12-15,000 xg for 15 min. Ultracentrifugation is best, however.
12. The supernatant will contain topo I and II activities. The type I activity can easily be assayed using the Topo I assay kit (see below) and the type II using kDNA and the TopoGEN Topo II assay kit. Usually, 107 to 108 cells extracted in this way should give large amounts of activity in 1 ul of extract; however, one should titrate over a wide range (from 1:100 dilution in TEMP to as much as 4 ul) to ensure that the reactions are not overloaded. Since the extract contains high salt (0.5M) take care not to poison the reaction with excessive amounts of the extract.