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# Top2 Chromatin Accessibility by Etoposide Cross-linking

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**Protocol status:** Working
**We use this protocol and it's working**

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**Keywords:** TOP2, DNA accessibility, chromatin

## Abstract

**The best way to determine TOP2 specific accessibility to chromatin is via etoposide treatment, which leads to a covalent cross-link between TOP2 proteins and DNA, that can only occur where TOP2 proteins have access to DNA. TOP2 cross-linking to DNA has previously been shown to require DNA accessibility and chromatin regulators that alter DNA accessibility (Dykhuizen and Hargreaves et al. Nature 2013, Miller et al. NSMB 2017).**

## Guidelines

Buffer A

- 25mM HEPES pH 7.0
- 25mM KCl
- 0.05mM EDTA
- 5mM MgCl2
- 10% Glycerol
- 0.1% NP-40

High Salt RIPA

- 50mM TRIS pH 7.8
- 500mM NaCl2
- 1% NP-40
- 0.1% DOC
- 0.1% SDS

1% SDS RIPA

- 50mM TRIS pH 7.8
- 150mM NaCl2
- 1% NP-40
- 0.1% DOC
- 1% SDS

Protease Inhibitors (1000x stock in DMSO all from Calbiochem)

- Chymostatin #230790 (10mg/ml)
- Leupeptin, Hemisulfate #108975 (10mg/ml)
- Pepstatin A, Synthetic #516481 (10mg/ml)

1 Cell Culture: 15m
Trypsinize and count cells
Transfer 0.75e6-1e6 cells to (4) different eppendorfs
Save (1) eppendorf for your input sample for step 4
Bring volumes to 1ml with media (If volume is already more than 1ml you can transfer to a 15ml conical and bring volume up to known amount where all cell lines are at the same volume)

2 Etoposide Cross-linking 1h
Add Etoposide (1:1000; final concentration of 100uM) for 0, 15 and 60 minutes
Add to 60' sample first, and then 15' sample 45' later
Rotate at RT for 60'

3 Washes 10m
Spin at 500g for 4 minutes at 4°C
Wash with PBS
Spin at 500g for 4 minutes at 4°C
Remove suppernatant

4 Buffer A -- Making Nuclei 15m
For all samples + Input
Resuspend in 1 ml Buffer A + Protease Inhibitors (1:1000) + DTT (1:1000; final concentration:)
Incubate on ice for 10'
Spin at 300g for 4 minutes
Aspirate out suppernatant
Spin breifly and remove remaining suppernatant

5 High salt RIPA extraction 25m
Resuspend nuclei in 50-100ul High salt RIPA with 500mM NaCl
Incubate on ice for 20'
Spin 12,000g for 5' at 4°C
Remove suppernatant (can save if you want)
Spin breifly and remove remaining suppernatant

6 Solubalize chromatin pellet 33m
Resuspend in 50ul 1% SDS RIPA + PIs + DTT + Benzonase (1:200)
Be sure to fully resuspend pellet by pipetting up and down ~20 times
Incubate at room temp for 10'
Incubate on ice for 20'
Spin 12,000g for 3 minutes and transfer suppernatant to a new tube (there should not be much if any of a visible chromatin pellet after spinning. If there is then try resuspending in a larger volume)

7 Proceed to Western Blot adding equal volumes of each sample