Chromatin Associated Protein Isolation

1. Make crude nuclear extract:

1.1. To cell pellet, add 125ul Buffer A (-triton), re-suspend cells.
1.2. Add 125ul Buffer A + 2X Triton (0.2%).
1.3. Incubate on ice 8 min.
1.4. Spin at 3800 RPM (1300g)
1.5. Remove supernatant (cytosol fraction)

2. Benzonase Treatment:

2.1. To pellet, add 75ul (adjust as needed) Buffer A + inhibitors + 0.1% Triton + benzonase (1ul/ml)
2.2. Incubate on ice 1-2 hours.

3. Denature and boil:

3.1. Add 1/3volume of 4X SDS-PAGE loading buffer with B-ME (ex. Add 25ul to 75ul of lysate)
3.2. Mix and boil for 5 min.

4. Quick spin sample to collect condensation before loading.

5. Bradford samples (take 1-2ul for Bradford—see separate protocol)

Buffer A
10mM Hepes 7.9
10mM KCl
1.5mM MgCl2
0.34M sucrose
10% glycerol
0.1% Triton (final)

Inhibitors (in -20):
200X cocktail (5ul per ml)
B-GP (10ul per ml)
PMSF (10ul per ml)

Benzonase located in -20 Antibodies box