**Nuclear cytoplasmic fractionation.**

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**Regents required:**

PBS

SDS

Benzonase

**Hypotonic buffer**

10 mM Hepes (pH 7.9)

10 mM KCl

0.1 mM EDTA

0.1 mM EGTA

1 mM dithiothreitol (DTT)

**high-salt buffer**

20 mM HEPES

400 mM NaCl

1 mM EDTA

1 mM EGTA

1 mM DTT

0.5% NP-40

**Steps:**

1. 500,000 BMDM’s were treated with DMSO or 0.5nM MLi-2 for 6 hours.
2. After the treatment cells were washed 3X in PBS.
3. The cells are harvested in 500 μl of ice-cold hypotonic buffer.
4. Then the cells are homogenized with 20 strokes of a Dounce homogenizer.
5. To 100μl of this homogenate SDS (1% final) and 25 U of Benzonase (Novagen) were added. This is used as total lysate.
6. The rest of the homogenate was spun at 4°C for 5 min at 14,000 rpm.
7. The supernatant (cytoplasmic fraction) was collected into a new tube.
8. The pellet (nuclear fraction) was resuspended in 200 μl of high-salt buffer and solubilized with SDS (1% final) in the presence of 25 U of Benzonase.
9. Protein concentration was measured with the BCA reagent (Thermo Scientific), and samples were subsequently analyzed by immunoblotting.