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Nuclear cytoplasmic fractionation

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We use this protocol and it's working

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Abstract

This protocol describes nuclear cytoplasmic fractionation.

Attachments

DOCX

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15KB

Materials

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

Troubleshooting

Nuclear cytoplasmic fractionation

6h 5m

1 Treat 500,000 BMDM's with DMSO or [M] 50 nanomolar (nM) MLi-2 for 06:00:00 . 6h

2 After the treatment, wash the cells 3X in PBS.

3 Harvest the cells in 500 µL of ice-cold hypotonic buffer.

4 Homogenize the cells with 20 strokes of a Dounce homogenizer.

5 To 100 µL of this homogenate, add SDS (1% final) and 25 U of Benzonase (Novagen). Use this as total lysate.

6 Spin the rest of the homogenate at 14000 rpm, 4°C, 00:05:00 . 5m

7 Collect the supernatant (cytoplasmic fraction) into a new tube.

8 Resuspend the pellet (nuclear fraction) in 200 µL of high-salt buffer and solubilize with SDS (1% final) in the presence of 25 U of Benzonase.

9 Measure protein concentration with the BCA reagent (Thermo Scientific), and subsequently analyze the samples by immunoblotting.