

Protocol for inducing MMP-9 expression

Use PMA to induce MMP-9 expression in cell lines, and prepare lysates for downstream analysis

In many cell lines, MMP9 is expressed at very low levels. However, treatment with phorbol myristate acetate (PMA) – a protein kinase C ligand – markedly stimulates MMP9 expression¹.

For analysis of MMP9 in cell lines, it may be necessary to first stimulate your cells with PMA to detect a signal.

Cell stimulation with PMA

The following protocol has been optimized for use with U937 cells.

1. Culture cells at 37°C in RPMI 1640 medium containing 2 mM glutamine and 10% fetal bovine serum (FBS).
2. When the cell culture reaches a density of 0.5–1x10⁶ cells/mL, transfer 20 mL (2x10⁻⁵) cells into a new flask or plate and add PMA to a final concentration of 10 ng/mL.
3. After incubation for 21 h at 37°C, add Brefeldin A to a final concentration of 3 µg/mL and incubate for a further 3 h.

Treatment with Brefeldin A inhibits protein secretion.

4. Collect cells by centrifugation, and wash with PBS.
5. Snap freeze the pellet with liquid nitrogen.

Cells can be stored at -80°C or immediately lysed for western blot analysis.

Cell lysis

1. Add 300 µL ice-cold lysis buffer to the frozen cell pellet.
2. Resuspend cells by gently pipetting.

If cells remain clumped together, vortex the tube for 10 s, incubate the tube on ice for 3 min, and attempt to resuspend again.

3. Incubate on ice for 10 min, then pipette the cells again to resuspend any remaining pelleted material.
4. Sonicate for three cycles of 40 s. If the cell pellet is still visible, repeat sonication.

A slight yellow color indicates good lysis.

5. Centrifuge the lysate at 8000 rcf for 10 min at 4°C to pellet the cell debris.
6. Transfer the supernatant to a fresh tube, being careful not to transfer any of the cell debris.

Lysate can either be stored at -80°C or used immediately for western blotting.

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Buffers

RIPA lysis buffer

- 150 mM sodium chloride
- 1.0% NP-40 or Triton X-100
- 0.5% sodium deoxycholate*
- 0.1% SDS (sodium dodecyl sulfate)
- 50 mM Tris, pH 8.0

Add proteinase inhibitor before use.

References

Roomi MW, Monterrey JC, Kalinovsky T, Rath M, Niedzwiecki A. Patterns of MMP-2 and MMP-9 expression in human cancer cell lines. *Oncol Rep.* 21, 1323–1333 (2009)