

Catalog No. 11984-050 50 X 1ml Reactions

Overview and Intended Use

Protocol for Preparing Adherent Cells for Nuclear Extraction.

1. Remove cell culture media by aspiration and wash cells twice with 2 – 10 ml of ice-cold PBS (not containing calcium or magnesium).
2. Adherent Cell Lysis Solution should be prepared in a pre-chilled container. Calculate the required amount of Adherent Cell Lysis Solution required using the chart below as a guide.

Culture Vessel	Volume of Adherent Cell Lysis Solution
6-well plate	500 µl/well
100mm X 20 mm Dish	2 ml/dish
T-25 Flask	500 µl/flask
T-75 Flask	2 ml/flask
T-175 Flask	4 ml/flask

3. Prepare the Complete Adherent Cell Lysis Solution according to the Table below.

Add the following for each 1ml of Adherent Cell Lysis Solution		
	µl to add	Final Concentration
Protease Inhibitor (<i>Sigma Cat#P8340</i>)	10	1x
*Ser/Thr Phosphatase Inhibitor (<i>Sigma Cat# P2850</i>)	10	1x
*Tyrosine Phosphatase Inhibitor (<i>Sigma Cat# P5726</i>)	10	1x
0.1M DTT (<i>Sigma Cat#D9779</i>)	10	1mM
10mM PMSF (<i>Sigma Cat#P7626</i>)	10	100µM

* Phosphatase inhibitors are recommended to preserve transcription factor activity, but may be omitted depending on the individual applications.

4. Add Complete Adherent Lysis Solution to cover cells, collect cells with a rubber policeman or cell scraper and transfer to a 15 ml conical tube.
5. Pellet the cells by centrifugation at 800 x g for 5 minutes at 4°C and remove the supernatant.
6. Use cells immediately to prepare nuclear extracts or freeze at –80°C.
7. If preparing for Marligen Multiplex Testing Services, freeze cells at –80°C and ship samples on dry ice to:

Attention: Transcription Factor Services
Marligen, Biosciences Inc.
2502 Urbana Pike
Ijamsville, MD 21754

For complete testing services ordering instructions please visit our website at:

<http://www.marligen.com/multiplex-testing-services.html>