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## Nuclear Extraction Kit

Catalog Number SK-0001

(For Research Use Only)

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### Introduction

The Nuclear Extraction Kit is used for preparation of nuclear extracts, which often need for studying transcription factors. The Nuclear Extraction Kit contains reagents for the preparation of 200 nuclear extracts from culture cells in 6-well plate and 50 nuclear extracts from cultured cells in 100-mm culture dishes.

### Materials provided with the kit

- 10X Buffer I (RT)  
Dilute 1 X Buffer before use with ddH<sub>2</sub>O
- 5x Buffer II (RT)  
Dilute 1 X Buffer before use with ddH<sub>2</sub>O
- DTT solution (-20 °C)
- Protease Inhibitor (-20 °C)

### Material required but not provided

- 1xPBS
- Centrifuge
- Cell scraper (for adherent cell types)
- Shaking platform

### Assay Procedure

10<sup>7</sup> cells usually yield 0.1-0.3 mg at 1-5µg/µl.

### Prepare from cell culture

#### I. For adhere cells

1. Wash the cells with 1xPBS
2. Prepare 1ml of Buffer I working reagent by mixing the following components  
1ml 1X buffer I  
10ul DTT solution  
10ul Protease inhibitor
3. Add Buffer I working reagent to the cells  
1ml/100mm dish (10<sup>7</sup> cells)  
250ul /well of 6-well plate (2x10<sup>6</sup> cells)
4. If the cell from tissue, add appropriate amount of buffer according to the cell number
5. Put culture dish(s) to an ice box, and rock at 200rpm for 10 minutes on a shaking platform
6. Release the cells from the dish using a sterile scraper. Transfer the cells to a 1.5ml microcentrifuge tube and centrifuge at 10000xg for 5 minutes at 4°C.
7. Discard the supernatant and keep the pellets on ice.

8. Prepare buffer II working reagent  
1ml 1x Buffer II  
10ul DTT solution  
10ul Protease inhibitor
9. Add 250 ul to the cell pellet from 100 mm culture dish (10<sup>7</sup> cells), and 50ul to the cell pellet from 6 - well plate (2x10<sup>6</sup> cells). (**Don't attempt to disperse the pellet**)
10. Place the tube on ice into an ice box, and rock at 200rpm on a platform for a hour
11. Centrifuge sample at 12000xg for 5 minutes at 4°C.
12. Transfer **supernatant** to a new tube. This is **your nuclear extract**.

#### II. For suspension cells

1. Transfer cells to a 1.5 ml or 15 ml centrifuge tube as appropriate and centrifuge at 500 x g for 5 minutes
2. Remove the culture media and wash cells by resuspending in 1 mL of cold 1X PBS followed by centrifugation at 500 x g for 5 minutes.
3. Proceed the procedure from step 2 in section I for adhere cells.

### Prepare from tissue

1. Cut 20-100 mg of tissue into small pieces and place in a microcentrifuge tube.
2. Wash tissue with PBS. Centrifuge tissue at 500 × g for 5 minutes.
3. Using a pipette, carefully remove and discard the supernatant, leaving cell pellet as dry as possible.
4. Homogenize tissue using a Dounce homogenizer or a tissue grinder in the 1.5ml-3ml of 1x buffer I with DTT and protease inhibitor until a single cell suspension is observed (by microscope). All of procedure must perform on ice.
5. Transfer homogenate to a fresh tube and spin at 500 x g for 5 minutes at 4°C.
6. Continue the procedure with the cell pellet at step 2 in section I. The amount of Buffer I is used based on the amount of tissue.  
**20mg tissue is equivalent to 2x10<sup>6</sup> cells. 100mg is equivalent to 10<sup>7</sup> cells .**