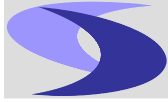

Nuclear Extraction Kit

Catalog Number SK-0001 **(For Research Use Only)**

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 to 1X Buffer I with ddH2O before use
- 5x Buffer II (RT)
  -Dilute to 1X Buffer II with ddH2O before use
- DTT solution (-20 ºC)
- Protease Inhibitor (-20 ºC)

Material required but not provided

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

Assay Procedure

10^7 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 1X PBS
2. Prepare 1ml of Buffer I working reagent by mixing the following the components
   1ml 1X Buffer I
   10ul DTT solution
   10ul Protease inhibitor
3. Add Buffer I working reagent to the cells
   1ml / 100mm dish (10^7 cells),
   or 250ul / well of 6-well plate ($2x10^6$ cells)
4. 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 12000rpm for 5 minutes at 4ºC.
7. Discard the supernatant **thoroughly and completely**. Keep the pellets on ice.
8. Prepare buffer II working reagent
   1ml 1X Buffer II
   10ul DTT solution
   10ul Protease inhibitor
9. Add Buffer II working reagents to the cells
   250ul / 100 mm culture dish (10^7 cells),
   or 50ul / 6 - well plate ($2x10^6$ cells).
   Note: Addition of Buffer II working reagents should be at least 5 times more than the volume/size of the nuclear pellet.
   (Don't attempt to disperse the pellet by pipetting up and down. Tap gently to mix the pellet and Buffer II working reagents)
10. Place the tube on ice into an ice box, and shake at 200rpm on a platform for two hours.
    (Lay tubes flat on ice on a shaking platform to allow complete contact of pellet and Buffer II solutions)
11. Centrifuge sample at 12000rpm for 5 minutes at 4ºC**.**
12. Transfer **supernatant** to a new tube. This is **your nuclear extract.**

II. For suspension cells

1. Transfer cells to a 1.5ml 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 3,000rpm for 5 munutes
3. Proceed the procedure from step 2 in section 1 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. Prepare Buffer I working reagent
   4ml 1X Buffer I
   40ul DTT solution
   40ul Protease inhibitor

5. Add 1.5ml-3ml of Buffer I working reagent and transfer the tissue into a Dounce homogenizer or a tissue grinder and homogenize tissue using until a single cell suspension is observed (by microscope). All procedures must perform on ice.
6. Transfer homogenate to a fresh tube and spin at 500 x g for 5 minutes at 4°C. Remove the supernatant, and add 1ml of Buffer I working reagent to resuspend the cell pellet in a 1.5ml microcentrifuge tube.
7. Lay the tube flat on the ice in an ice-box and rock at 200rpm for 10 minutes on a shaking platform.
8. Transfer the cells to a 1.5ml microcentrifuge tube and centrifuge at 10,000rpm for 5 minutes at 4°C.
9. Discard the supernatant **thoroughly and completely**. Keep the pellets on ice.
10. Prepare buffer II working reagent
    1ml 1X Buffer II
    10ul DTT solution
    10ul Protease inhibitor
11. Add Buffer II working reagents to the cells
    The amount of Buffer II is used based on the amount of tissue. 100ul of Buffer II working reagent is added to the nuclear pellets from 20mg tissue (equivalent to $2x10^6$ cells). 250ul of Buffer II working reagent is added to the nuclear pellets from 100mg (equivalent to 10^7 cells).
    Note: Addition of Buffer II working reagents should be at least 5 times more than the volume/size of the nuclear pellet.
    (Don't attempt to disperse the pellet by pipetting up and down. Tap gently to mix the pellet and Buffer II working reagents)
12. Place the tube on ice into an ice box, and shake at 200rpm on a platform for two hours.
    (**Lay tubes flat on ice to allow complete contact of pellet and Buffer II solutions)**
13. Centrifuge sample at 12,000rpm for 5 minutes at 4°C**.**
14. Transfer **supernatant** to a new tube. This is **your nuclear extract.**