Signosis
Innovative Plate Assay Solutions

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. The kit can also be used for nuclear extraction of soft tissue samples (brain, kidney, liver, etc), however there may be additional steps required to release cells from tissues.

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 1-5 µg/µl.

Prepare from cell culture

I. For adhere cells

1. Wash the cells with 1X PBS
2. Prepare 1 ml of Buffer I working reagent by mixing the following the components
   1 ml 1X Buffer I
   10 µl DTT solution
   10 µl Protease inhibitor
3. Add Buffer I working reagent to the cells
   1 ml / 100 mm dish (10^7 cells),
   or 250 µl / well of 6-well plate ($2x10^6$ cells)
4. Place culture dish(s) in an ice box, and rock at 200 rpm for 10 minutes on a shaking platform
5. Release the cells from the dish using a sterile scraper. Transfer the cells to a 1.5 ml microcentrifuge tube and centrifuge at 12000 rpm for 5 minutes at 4ºC.
6. Discard the supernatant **thoroughly and completely**. Keep the pellets on ice.
7. Prepare buffer II working reagent
   1 ml 1X Buffer II
   10 µl DTT solution
   10 µl Protease inhibitor
8. Add Buffer II working reagents to the cells
   250 µl / 100 mm culture dish (10^7 cells),
   or 50 µl / 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.
   (Do <u>NOT</u> attempt to disperse the pellet. Tap gently to allow the pellet to float in the Buffer II working reagents.)
9. Place the tube on ice into an ice box and shake at 200 rpm 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)
10. Centrifuge sample at 12000 rpm for 5 minutes at 4ºC.
11. 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 3,000 rpm for 5 minutes
3. Proceed the procedure from step 2 in section 1 for adhere cells.

Prepare from tissue

1. Cut 20-100 mg of soft tissue into small pieces and place in a 1.5 ml microcentrifuge tube.
2. Wash tissue with 1X PBS. Centrifuge tissue at 500 × *g* for 5 minutes.
3. Using a pipette, carefully remove and discard the supernatant, leaving pellet as dry as possible.

Note: Some tissues such as cornea or nerve fibers may require mechanical dissection and digestion with enzymes such as collagenase and/or hyaluronidase before proceeding with step 4.

4. Prepare Buffer I working reagent
   4 ml 1X Buffer I
   40 µl DTT solution
   40 µl Protease inhibitor
5. Add 1.5-3 ml of Buffer I working reagent and transfer the tissue into a Dounce homogenizer or a tissue grinder and homogenize tissue until a single cell suspension is observed (by microscope). All procedures must be performed on ice.
6. Transfer homogenate to 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 1.5 ml microcentrifuge tube.
7. Lay the tube flat on the ice in an icebox and rock at 200 rpm for 10 minutes on a shaking platform.
8. Centrifuge at 10,000 rpm for 5 minutes at 4°C.
9. Discard the supernatant **thoroughly and completely**. Keep the pellets On ice.
   Optional: For better cell lysis, repeat steps 4-9 one more time.
10. Prepare Buffer II working reagent
    1 ml 1X Buffer II
    10 µl DTT solution
    10 µl Protease inhibitor
11. Add Buffer II working reagents to the cells.
    The amount of Buffer II is used based on the amount of tissue. 100 µl of Buffer II working reagent is added to the nuclear pellets from 20mg tissue (equivalent to $2x10^6$ cells).
    250µl 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.
    (Do <u>NOT</u> attempt to disperse the pellet. Tap gently to allow the pellet to float in the Buffer II working reagents.)
12. Place the tube on ice into an ice box, and shake at 200 rpm 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 12,000 rpm for 5 minutes at 4°C**.**
14. Transfer **supernatant** to a new tube. This is **your nuclear extract.**

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We suggest store nuclear extract in aliquots in the -80°C. It minimizes any damage that could be caused by thawing and refreezing. Usually, it can last for at least 6 months.