Product Information

NuCLEAR™ Extraction Kit

For mammalian tissue or cultured cells

NXTRACT

Product Description

The preparation of an extract from nuclei is often the first step in studying nuclear proteins and their interactions. The resulting preparation can be used directly in the Electrophoresis Mobility Shift Assay (EMSA), foot printing analysis, transcription assays,[1-4] or as a starting point for the purification of regulatory proteins.

The general procedure for the nuclear protein extraction method is to allow cells to swell with hypotonic buffer. The cells are then disrupted, the cytoplasmic fraction is removed, and the nuclear proteins are released from the nuclei by a high salt buffer.[1-3]

This protocol is written for the extraction of functional, crude nuclear proteins from various cell types. The NuCLEAR™ Extraction Kit has been tested for HeLa, CHO, COS, PC-12, Jurkat, and Bovine Aorta Endothelial Cells (BAEC). Nuclear proteins can be extracted either from a small number of cells (10^6–10^7 cells, from 20-100 µL of packed cell volume), or from a much larger number of cells (10^8–10^{10} cells, from ~1 mL of packed cell volume).

Nuclear proteins can be extracted from the following fresh or frozen tissues: mouse brain and lung, rat liver, and rabbit muscle. The protein extracts are suitable for the detection of DNA-protein interaction using a gel-shift assay, DNase I foot printing analysis, and similar techniques.

Several theses[5] and dissertations[6-24] have cited use of product NXTRACT in their protocols.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

The reagents provided are sufficient for either 100 extractions of 100 µL of packed cell volume, or 10 extractions of 1 mL of packed cell volume.

- 10× Lysis Buffer, hypotonic, 7 mL (Component L9161): 100 mM HEPES (pH 7.9), with 15 mM $MgCl_2$ and 100 mM KCl
- 5× Lysis Buffer, isotonic, 14 mL (Component L9036): 50 mM Tris HCl (pH 7.5), with 10 mM $MgCl_2$, 15 mM $CaCl_2$, and 1.5 M Sucrose
- Extraction Buffer, 10 mL (Component E2525): 20 mM HEPES (pH 7.9), with 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol
- 3× Dilution and Equilibration Buffer, 90 mL (Component D0187): 60 mM HEPES (pH 7.9), with 4.5 mM $MgCl_2$, 0.6 mM EDTA, 30 mM KCl, and 75% (v/v) glycerol
- Dithiothreitol (DTT), 0.4 mL (Component D7059): 1 M DTT in deionized water
- Protease Inhibitor Cocktail, 1 mL (Cat. No. P8340), which contains:
  - 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF)
  - Pepstatin A
  - Bestatin
  - Leupeptin
  - Aprotinin
  - trans-Epoxysuccinyl-L-leucyl-amido(4-guanidino)-butane (E-64)
- IGEPAL® CA-630 10% Solution, 4 mL (Component I5402): 10% IGEPAL® CA-630 in deionized water

Reagents and Equipment Required for Extraction (Not provided)

- Centrifuge tubes
- Centrifuge with A-4-62 rotor, or equivalent (optional)
- Microcentrifuge
- Glass tissue homogenizer, grind tube and type B pestle (optional)
- Microscope
- Syringes, 1 mL capacity
- Precision Glide Needles, SS, 27 gauge (optional)
- Microscope slides, Cat. No. S8902
- Micro cover glass, Cat. No. C9802
- Cell scrapers, Cat. No. CLS3010
- Dulbecco's phosphate-buffered saline (D-PBS), Cat. No. D8537
- Trypan Blue solution, Cat. No. T8154

Reagents and Equipment Recommended for Salt Removal

(Not provided)

- Sephadex® PD-10 columns (desalting column for 2.5 mL of solution): Cat. No. GE17-0851-01
- 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF): Cat. No. A8456

Storage/Stability

This kit is shipped on dry ice. Long-term storage at –20 °C is recommended. The DTT solution and the protease inhibitor cocktail must be stored at –20 °C and should be added to buffers immediately before use. All the buffer solutions may be stored at 2-8 °C for several weeks. The 10% IGEPAL® CA-630 Solution may be stored long-term at 2-8 °C.

Procedure

General Notes

- Perform all steps at 2-8 °C.
- Use pre-cooled buffers and equipment.
- Ensure all the solutions are defrosted and homogeneous.
- All centrifugations are done at 4 °C with pre-cooled rotors.
- The final concentration of DTT in the solutions should be 1 mM.
- The protease inhibitor cocktail should be diluted 100-fold in the final solutions.

Nuclear Protein Extraction from 100 µL of packed cell volume using a detergent (IGEPAL® CA-630)

Calculate accordingly for different packed cell volumes.

1. Dilute the 1 M DTT solution with deionized, sterile water to a concentration of 0.1 M. For small-scale preparations (<100 µL total), the 1 M DTT stock solution should be diluted to 0.01 M.
2. Prepare 1× Lysis Buffer, hypotonic, from the 10× Lysis Buffer, hypotonic, by diluting 10-fold with sterile, deionized water.
    a. For fragile cells, use 1× Lysis Buffer, isotonic, prepared from the 5× Lysis Buffer, isotonic, to replace the 1× Lysis Buffer, hypotonic.
    b. To 500 µL of 1× Lysis Buffer (either hypotonic or isotonic), add 5 µL of the prepared 0.1 M DTT solution and 5 µL of the protease inhibitor cocktail.
3. Collect cells.

    Adherent cells from 70-90% confluent monolayer culture

    a. Remove the growth medium from the cells.
    b. Rinse the cells twice with PBS, being careful not to dislodge any of the cells.
    c. Discard the PBS.
    d. Scrape the cells using fresh PBS into an appropriate conical centrifuge tube.
    e. Centrifuge for 5 minutes at 450 x g.
    f. Decant and discard the supernatant.

    Cells in suspension

    a. Collect the cells into an appropriate conical centrifuge tube.
    b. Centrifuge for 5 minutes at 450 × g.
    c. Decant and discard the supernatant.
    d. Wash the cells twice:
    e. Resuspend the cell pellets in PBS.
    f. Centrifuge for 5 minutes at 450 x g.
    g. Decant and discard the supernatant.
    h. Estimate the packed cell volume (PCV).
    i. Add 500 µL (5× PCV) of 1× Lysis Buffer (including DTT and protease inhibitors) to 100 µL of PCV. Resuspend the cell pellet gently. Avoid foam formation.

- If working with small volumes, the suspended cells may be transferred to a microcentrifuge tube.

4. Incubate the packed cells in the selected lysis buffer on ice for 15 minutes, allowing the cells to swell.
    a. Take several microliters of the cells in the lysis buffer.
    b. View them under the microscope.
    c. If massive cell lysis is detected under the microscope or a gelatinous mass is observed, the cells may be fragile.
    d. In this case, use the Lysis Buffer, isotonic, for cell lysis.
    e. The researcher may consider eliminating the incubation step.
5. To the swollen cells in lysis buffer, add the 10% IGEPAL® CA-630 solution to a final concentration of 0.6% (6 µL per 100 µL of mixture). Vortex vigorously for 10 seconds.
6. Centrifuge immediately for 30 seconds at 10,000–11,000 × g.
    a. To assess the degree of lysis, take a sample of the suspended cells before centrifugation.
    b. View the nuclei under the microscope.
    c. Lysis can be observed by the addition of the Trypan Blue solution to an aliquot of cells. The dye is excluded from the intact cells but stains the nuclei of lysed cells.
    d. If lysis of nuclei is observed under the microscope or if a gelatinous mass is observed, lyse the cells with a lower final concentration of IGEPAL® CA-630.
    e. If cells are not lysed, increase the final percentage of IGEPAL® CA-630 in the resuspended cells (Step 7).
7. For fragile cells:
    - Use lower concentrations of IGEPAL® CA-630.
    - Avoid vortexing the cells.
    - Centrifuge at a slower speed.
8. Transfer the supernatant to a fresh tube. This fraction is the cytoplasmic fraction.
9. Add 1 µL of the prepared 0.1 M DTT solution and 1 µL of the protease inhibitor cocktail to 98 µL of the Extraction Buffer. If it is necessary to extract the proteins of interest at a lower salt concentration, dilute the Extraction Buffer with 1× Dilution and Equilibration Buffer.

Notes on salt concentration:

- The salt concentration in the Extraction Buffer is 0.42 M, a commonly used extraction condition.
- In rare cases, a lower or a higher salt concentration may be needed for a better extraction of a particular protein.
- In such cases, either:
    - Dilute the Extraction Buffer with the 1× Dilution and Equilibration Buffer, or:
    - Add NaCl to the Extraction Buffer to reach the desired salt concentration.

10. Resuspend the crude nuclei pellet in ~70 µL (2/3× PCV) of Extraction Buffer containing the DTT and protease inhibitor cocktail.
11. Mount the tube on a vortex mixer. Agitate at medium to high speed for 15-30 minutes. Avoid foam formation.
12. Centrifuge for 5 minutes at 20,000–21,000 × g.
13. Transfer the supernatant to a clean, chilled tube.
14. Snap-freeze the supernatant in aliquots with liquid nitrogen. Store at –70 °C.

Nuclear Protein Extraction from 200 µL of packed cell volume without the use of a detergent

General Notes

- Calculate accordingly for different packed cell volumes.
- Detergents can interfere with the activity or binding of the extracted proteins. Therefore, a procedure for nuclear protein extraction without the use of a detergent is included here.
- This protocol describes the preparation of crude nuclear extracts using a syringe or a glass tissue homogenizer.
- The procedure requires at least 100 µL of PCV.
- Use of a syringe is recommended for small-scale preparations (0.1 mL–1 mL). Passage of > 1 mL through a syringe may cause difficulties because of the needle gauge size.

1. Dilute the 1 M DTT solution to 0.1 M with deionized, sterile water.
2. Prepare 1× Lysis Buffer, hypotonic.
    - For protein extraction from fragile cells, prepare 1× Lysis Buffer, isotonic, to replace the 1× Lysis Buffer, hypotonic.

- To 1,400 µL of 1× Lysis Buffer (hypotonic or isotonic), add 14 µL of the prepared 0.1 M DTT solution and 14 µL of the protease inhibitor cocktail.

3. Collect cells:

   Adherent cells from 70-90% confluent monolayer culture

   a. Remove the growth medium from the cells.
   b. Rinse the cells twice with PBS, being careful not to dislodge any of the cells.
   c. Discard the PBS.
   d. Scrape the cells using fresh PBS into an appropriate conical centrifuge tube.
   e. Centrifuge for 5 minutes at 450 × g.
   f. Decant and discard the supernatant.

   Cells in suspension

   a. Collect the cells into an appropriate centrifuge conical tube.
   b. Centrifuge for 5 minutes at 450 x g.
   c. Decant and discard the supernatant.
   d. Wash cells twice:
   e. Resuspend the cell pellets in PBS.
   f. Centrifuge for 5 minutes at 450 x g.
   g. Decant and discard supernatant.

4. Estimate the packed cell volume (PCV).
5. Add 1 mL (5× PCV) of 1× Lysis Buffer (including DTT and protease inhibitors) to 200 µL of PCV.
6. Resuspend the cell pellet gently. Avoid foam formation.
   - If working with small volumes, the suspended cells may be transferred to a microcentrifuge tube.
   - Incubate the packed cells in lysis buffer for 15 minutes, allowing the cells to swell.
7. Centrifuge the suspended cells for 5 minutes at 420 × g. Decant supernatant and resuspend the pellet of packed cells in 400 µL (2× PCV) of the 1× Lysis Buffer.
8. Cell disruption:

   Using a glass tissue homogenizer

   a. Transfer the cells into a glass tissue grind tube.
   b. Grind on ice slowly with five up-and-down strokes using a type B pestle. Avoid foam formation.

   Alternative: using a syringe with a narrow-gauge (No. 27) hypodermic needle

   a. Fill the syringe with 1× Lysis Buffer. The syringe plunger is used to displace the buffer as fully as possible. This removes all the air from the syringe and prevents excess air from being pumped into the cell suspension during lysis.
   b. Draw the cell suspension slowly into the syringe.
   c. Then eject with a single rapid stroke.
   d. Repeat five times.

   Notes:

   - The number of strokes needed (using the tissue homogenizer or the syringe) varies between cell lines.
   - Start with 5 strokes.
   - Then check lysis under the microscope. Lysis should be 80-90%.
   - If the lysis is not sufficient, perform several more strokes until lysis is complete.
   - Lysis can be observed by the addition of the Trypan Blue solution to an aliquot of cells. The dye is excluded from the intact cells, but stains the nuclei of lysed cells.
   - If nuclear lysis or clumps of nuclei are visualized, or if a gelatinous mass is observed, the cell disruption was too vigorous, or too many strokes were performed.

9. Centrifuge the disrupted cells in suspension for 20 minutes at 10,000-11,000 × g.
10. Transfer the supernatant to a fresh tube. This fraction is the cytoplasmic fraction.
11. Add 1.5 µL of the prepared 0.1 M DTT solution and 1.5 µL of the protease inhibitor cocktail to 147 µL of the 1× Extraction Buffer. If the proteins of interest are extracted at a lower salt concentration, dilute the Extraction Buffer with the 1× Dilution and Equilibration Buffer.

    Notes on salt concentration:

    - The salt concentration in the Extraction Buffer is 0.42 M, a commonly used extraction condition.
    - In rare cases, a lower or a higher salt concentration may be needed for a better extraction of a particular protein.
    - In such cases, dilute the Extraction Buffer with the 1× Dilution and Equilibration Buffer or add NaCl to the Extraction Buffer to reach the desired salt concentration.

12. Resuspend the crude nuclei pellet in ~140 µL (2/3× PCV) of Extraction Buffer containing the DTT and protease inhibitors. If the procedure is being performed with a tissue homogenizer, it is recommended to give 10 more strokes at this point.
13. Shake gently for 30 minutes.
14. Centrifuge for 5 minutes at 20,000-21,000 × g.
15. Transfer the supernatant to a clean, chilled tube.
16. Snap-freeze the supernatant in aliquots with liquid nitrogen. Store at –70 °C.

Nuclear protein extraction from 100 mg of tissue

Calculate accordingly for different tissue weights.

1. Dilute the 1 M DTT solution to 0.1 M with deionized, sterile water.
2. Prepare 1× Lysis Buffer:
3. For tissues internally tested, the hypotonic buffer works better than the isotonic buffer. Therefore, it is recommended to prepare 1× Lysis Buffer, hypotonic. If the tissue is found to be too fragile, one can use the 1× Lysis Buffer, isotonic.
4. Add 10 µL of the prepared 0.1 M DTT solution and 10 µL of the protease inhibitor cocktail to 1000 µL of 1× Lysis Buffer.
5. Rinse the tissue twice with PBS buffer. Discard the PBS.
6. Resuspend the tissue gently in 1,000 µL (5× PCV) of the 1× Lysis Buffer (containing DTT and protease inhibitors).
7. Homogenize the tissue until >90% of the cells are broken and nuclei are visualized under the microscope.
8. Centrifuge the disrupted cells in suspension for 20 minutes at 10,000-11,000 × g.
9. Transfer the supernatant to a fresh tube. This fraction is the cytoplasmic fraction.
10. Add 1.5 µL of the prepared 0.1 M DTT solution and 1.5 µL of the protease inhibitor cocktail to 147 µL of the Extraction Buffer.

    Note: If one wishes to extract nuclei proteins with a lower salt concentration, the Extraction Buffer may be diluted with 1× Dilution and Equilibration buffer.
11. Resuspend the crude nuclei pellet in ~140 µL (2/3× PCV) of Extraction Buffer containing DTT and protease inhibitor. At this stage, a short homogenization can be performed to facilitate nuclear extraction.
12. Shake gently for 30 minutes.
13. Centrifuge for 5 minutes at 20,000-21,000 × g.
14. Transfer the supernatant to a clean, chilled tube.
15. Snap-freeze the supernatant in aliquots with liquid nitrogen. Store at –70 °C.

Salt Removal

The nuclear proteins extracted according to the protocol are suspended in Extraction Buffer, a high salt buffer. Usually, the extracted proteins are highly concentrated, and can be diluted with a low salt buffer (1× Dilution and Equilibration Buffer). Since small amounts of the concentrated extract in a high salt buffer are sufficient for analysis by EMSA, foot printing, and similar assays, the salt dilution occurs naturally in the reaction tube itself.

If salts interfere with further experiments, removal of salts may be performed rapidly using desalting gel-filtration columns (see Reagents and Equipment Recommended for Salt Removal). The desalting columns require equilibration with 1× Dilution and Equilibration Buffer supplemented with DTT. The protease inhibitor cocktail should be added to the eluted protein fraction.

The salts may also be removed by dialysis of the nuclear extracts against a dialysis buffer that is similar to the 1× Dilution and Equilibration Buffer, containing a final concentration of 1 mM DTT and protease inhibitor cocktail or 0.5 mM AEBSF.

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NXTRACTpis Rev 07/23 NDH,EB,CMH,ES,GCY,MAM