ab113474 – Nuclear Extraction Kit

For the preparation of nuclear extracts from mammalian cells and tissue samples
View kit datasheet: www.abcam.com/ab113474
(use www.abcam.cn/ab113474 for China, or www.abcam.co.jp/ab113474 for Japan)

This product is for research use only and is not intended for diagnostic use

Storage and Stability: Store kit at +4°C immediately upon receipt.

Materials Supplied:

Item	100 Tests	Storage Condition
10X Pre-Extraction Buffer	10 mL	4°C
Extraction Buffer	10 mL	4°C
1000X DTT Solution	110 µL	4°C
1000X Protease Inhibitor Cocktail (PIC)	110 µL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Desktop centrifuge (up to 14,000 rpm) capable of 4°C
- Sonication device
- Dounce homogoniser
- Pipettes and pipette tips
- 1.5 mL microcentrifuge tubes
- Trypsin/EDTA
- Vortex-mixer

Limitations

- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between reagent additions.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

Reagent Preparation

1X Pre-Extraction Buffer

1. Dilute 10X Pre-Extraction Buffer with distilled water at a 1:10 ratio (e.g. 1 mL of 10X Pre-Extraction Buffer + 9 mL of distilled water).
2. Add 10 µL of DTT Solution and 10 µL PIC to ice cold 1X Pre-Extraction Buffer (1X) at a 1:1000 ratio.

Cell Pellet Preparation

For Monolayer or Adherent Cells

1. Grow cells to 70-80% confluency on a culture plate or flask (about 2-5 x 10^6 cells for a 100 mm plate). Remove the growth medium and wash cells with PBS twice and then remove PBS.
2. Add 1 mL of fresh PBS per 20 cm2 area (e.g. add 3 mL of PBS to a 100 mm plate), and scrape cells into a 15 mL conical tube.
    a. Alternative Option: detach cells with trypsin/EDTA and collect cells into a 15 mL conical tube. Count cells in a hemacytometer.
3. Centrifuge the cells for 5 minutes at 1000 rpm and discard the supernatant.
4. Resuspend cell pellet in 100 µL of 1X Pre-Extraction Buffer per 10^6 cells, and transfer to a micro-centrifuge vial.
5. Incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge the preparation for 1 minute at 12,000 rpm.
6. Carefully remove the cytoplasmic extract from the nuclear pellet. (The cytoplasmic protein fraction may be quantified and used for downstream applications.)

For Suspension Cells

1. Grow cells to 2 × 10^6/mL and collect the cells into a 15 mL conical tube.
2. Centrifuge the cells for 5 minutes at 1000 rpm and discard the supernatant. Wash cells with PBS once by centrifugation for 5 minutes at 1000 rpm. Discard the supernatant.
3. Re-suspend cell pellet in 100 µL of 1X Pre-Extraction Buffer per 10^6 cells and transfer to a microcentrifuge vial.
4. Incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge the preparation for 1 minute at 12,000 rpm.
5. Carefully remove the cytoplasmic extract from the nuclear pellet. (The cytoplasmic protein fraction may be quantified and used for downstream applications.)

Tissue Samples

1. Weigh the tissue and cut it into small pieces. Place tissue pieces in a clean homogenizer.

2. Add 5 mL of 1X Pre-Extraction Buffer containing 5 µL of DTT Solution per gram of tissue, and homogenize tissue pieces (50-60 strokes).
3. Incubate on ice for 15 minutes and centrifuge for 10 minutes at 12,000 rpm at 4°C.
4. Remove the supernatant.

Assay Procedure

Nuclear Extract Preparation

1. Add DTT Solution and PIC to Extraction Buffer at a 1:1000 ratio. Add 2 volumes of Extraction Buffer containing DTT and PIC to nuclear pellet (about 10 µL per 10^6 cells or per 2 mg of tissue). Incubate the extract on ice for 15 minutes with vortex (5 seconds) every 3 minutes. The extract (especially tissue extract) can be further sonicated for 3 x 10 seconds to increase nuclear protein extraction.
2. Centrifuge the suspension for 10 minutes at 14,000 rpm at 4°C and transfer the supernatant into a new microcentrifuge vial.
3. Measure the protein concentration of the nuclear extract.

Note: It is advised to use ab119216 Optiblot Bradford Reagent to measure the protein concentration of the nuclear extract due to its superior compatibility with interfering substances such as DTT and detergents in extraction buffer.

4. Use immediately or aliquot and freeze the supernatant at -80°C until further use. Avoid multiple freeze/thaw cycles.

Troubleshooting

Low yield of nuclear proteins

Possible Cause	Suggestion
Insufficient amount of samples	To obtain the best results, the amount of samples should be 2 x10^6 to 5x10^6 cells, or at least 10 mg of tissue
Cell pellets were not disrupted after addition of Pre-Extraction Buffer	Ensure that all reagents have been added with the correct volume and in the correct order based on the sample amount
	Check for cell lysis under a microscope after addition of Pre-Extraction Buffer and incubation on ice
	Ensure that the cell or tissue species are compatible with this extraction procedure
Incomplete lysis of cells or nucleus because too little buffer was used or lysis time was too short	Ensure that enough extraction buffer is used at each step of the protocol. Increase incubation time on ice to ensure complete cell lysis
Incomplete lysis of nucleus	Perform the sonication step to increase the yield of nuclear extracts. If the sonication step was performed, increase the sonication time or number of cycles to increase the yield of nuclear extracts
Lysis or extraction reagents have expired. Expired reagents may cause inefficient extraction	Ensure that the kit has not exceeded the expiration date
Incorrect temperature and/or insufficient incubation time during extraction	Ensure the incubation time and temperatures described in the protocol are followed

Low/no activity of nuclear enzymes in downstream activity assay

Possible Cause	Suggestion
Improper starting material	The enzyme activity of nuclear extracts from frozen tissue may be much lower than that from fresh tissues. We recommend using fresh cells or tissue whenever possible because of the risk of significantly reduced enzyme activity from frozen samples. Nuclear Extracts should be stored at -80°C (3-6 months). Avoid multiple freeze/thaw cycles
	This kit is not recommended for use with plant samples.

When performing protein concentration measurement: 1) the blank and samples are all saturated; and 2) the blank and samples turned a dark purple color immediately upon adding nuclear extracts or final working buffer for blank

Possible Cause	Suggestion
The protein quantification assay is not compatible with DTT present in the final working buffer.	1) Use a protein quantification assay that is reducing agent compatible. 2) Measure nuclear protein concentration before adding DTT. After the protein concentration has been measured the DTT can be added for storage purposes. 3) Perform a Bradford Assay for nuclear protein measurement.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)