ab113477 – Nuclear (Nucleic Acid-Free) Extraction Kit

Instructions for Use

For the preparation of nuclear (nucleic acid-free) extracts from mammalian cells and tissue samples.

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

Version 2a Last Updated 26 May 2020

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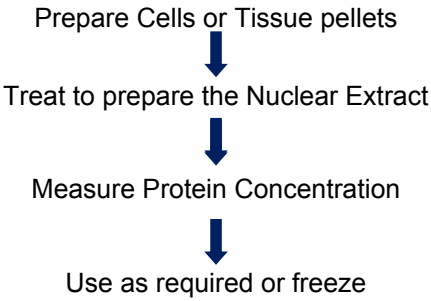
1. BACKGROUND

ab113477 provides the simple and selective method for extracting nuclear proteins used for a variety of applications. These applications may include western blotting, protein-DNA binding assays, and others requiring optimized or nucleic acid-free nuclear proteins. The Nuclear Extraction Kits are also specifically designed to meet the requirements of nuclear extracts used in various assays. The Nuclear Extraction Kits can be used to extract nuclear proteins from mammalian cells and tissue samples. The Nuclear Extraction Kits include ab113474 for regular nuclear extraction and ab113477 for nucleic acid-free nuclear extraction. They can be finished within 60 minutes.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

Aliquot Extraction Pre-Cleaner and Extraction Cleaner on arrival.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if Buffers contain salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

5. MATERIALS SUPPLIED

Item	100 Tests	Storage Condition (Before Preparation)
10X Pre-Extraction Buffer	10 mL	4°C
Extraction Buffer	10 mL	4°C
Extraction Pre-Cleaner	1 mL	-20°C
Extraction Cleaner	100 µL	-20°C
1000X DTT Solution	100 µL	4°C
1000X Protease Inhibitor Cocktail (PIC)	100 µL	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Desktop centrifuge (up to 14,000 rpm)
- Dounce Homogoniser
- Pipettes and pipette tips
- 1.5 mL microcentrifuge tubes
- 15 mL conical tubes
- Distilled water
- PBS

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- 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

8. 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.**

9. REAGENT PREPARATION

Prepare fresh reagents from the materials supplied for each use of the assay kit. Do not store reconstituted reagents.

9.1 **1X Pre-Extraction Buffer**

9.1.1 Dilute 10X Pre-Extraction Buffer with distilled water at a 1:10 ratio (ex: 1 mL of 10X Pre-Extraction Buffer + 9 mL of distilled water).

9.1.2 Add 10 µL of DTT Solution and 10 µL PIC to ice cold 1X Pre-Extraction Buffer (1X) at a 1:1000 ratio.

9.2 **Nuclear Extract Solution**

9.2.1 Add 1 µL of DTT Solution and 1 µL PIC to 1 mL of Extraction Buffer (1:1000 ratio).

9.2.2 Add 110 µL Extraction Pre-Cleaner to solution already prepared in step 9.2.1 (1:10 ratio).

10. SAMPLE PREPARATION

10.1 **For Monolayer or Adherent Cells**

10.1.1 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.

10.1.2 Add 1 mL of fresh PBS per 20 cm^2 area (e.g., add 3 mL of PBS to a 100 mm plate), and scrape cells into a 15 mL conical tube.
(Alternative Option: detach cells with trypsin/EDTA and collect cells into a 15 mL conical tube. Count cells in a hemacytometer.)

10.1.3 Centrifuge the cells for 5 minutes at 1000 rpm and discard the supernatant.

10.1.4 Resuspend cell pellet in 100 µL of 1X Pre-Extraction Buffer per 10^6 cells, and transfer to a micro-centrifuge vial.

10.1.5 Incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge the preparation for 1 minute at 12,000 rpm.

10.1.6 Carefully remove the cytoplasmic extract from the nuclear pellet.

10.2 **For Suspension Cells**

10.2.1 Grow cells to 2 × 10^6/mL and collect the cells into a 15 mL conical tube.

10.2.2 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.

10.2.3 Re-suspend cell pellet in 100 µL of 1X Pre-Extraction Buffer per 10^6 cells and transfer to a microcentrifuge vial.

10.2.4 Incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge the preparation for 1 minute at 12,000 rpm.

10.2.5 Carefully remove the cytoplasmic extract from the nuclear pellet.

10.3 **Tissue Samples**

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

10.3.2 Add 5 mL of 1X Pre-Extraction Buffer (Step 9.1.1) containing 5 µL of DTT Solution per gram of tissue, and homogenize tissue pieces (50-60 strokes).

10.3.3 Incubate on ice for 15 minutes and centrifuge for 10 minutes at 12,000 rpm at 4°C.

10.3.4 Remove the supernatant.

11. ASSAY PROCEDURE

Nuclear Extract Preparation

11.1 Add 2 volumes of Nuclear Extract Solution 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.

11.2 Centrifuge the suspension for 10 minutes at 14,000 rpm at 4°C and transfer the supernatant into a new microcentrifuge vial.

11.3 Add Extraction Cleaner to the supernatant at a 1:100 ratio (ex: add 10 µL of Extraction Cleaner to 990 µL of the supernatant and incubate for 15-20 minutes at room temperature.

11.4 Centrifuge the suspension for 1 minute at 14,000 rpm at 4°C and transfer the supernatant into a new microcentrifuge vial.

11.5 Measure the protein concentration of the nuclear extract.

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

12. NOTES

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