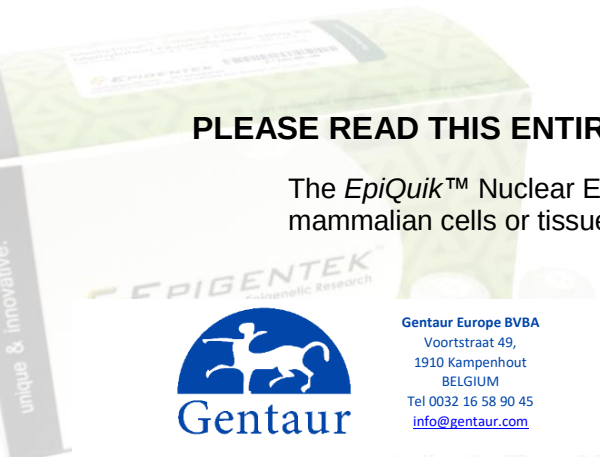
EpiQuik™ Nuclear Extraction Kit I

Base Catalog # OP-0002



PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Nuclear Extraction Kit is suitable for fast preparation of nuclear extracts from mammalian cells or tissue samples.

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KIT CONTENTS

Components	100 extractions OP-0002-1	Storage Upon Receipt
NE1 (10X Pre-Extraction Buffer)	10 ml	4˚C
NE2 (Extraction Buffer)	10 ml	4˚C
PIC (1000X Protease Inhibitor Cocktail)	110 µl	4˚C
DTT Solution (1M)	110 µl	4˚C
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SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C. Upon receipt, store all components at 4°C. The kit is stable for up to 1 year from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- PBS
- Trypsin/EDTA
- Desktop centrifuge (up to 14,000 rpm) capable of 4˚C
- Distilled or Deionized Water
- Vortex mixer
- Dounce homogenizer
- Sonication device
- 1.5 ml micro-centrifuge tubes

GENERAL PRODUCT INFORMATION

Quality Control: Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Usage Limitation: The *EpiQuik*™ Nuclear Extraction Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: *EpiQuik*™ is a trademark of Epigentek Group Inc.

A BRIEF OVERVIEW

The EpiQuik™ Nuclear Extraction Kit is a complete set of optimized reagents to provide a simple and selective method for isolating nuclear proteins used for a variety of applications in just 60 minutes. These applications may include Western blot, protein-DNA binding assays,

nuclear enzyme assays, and any other procedures requiring optimized nuclear proteins. The protocol is fast and easy-to-use, and also isolates very abundant yields of nuclear extract from mammalian cells or tissue samples. The kit is also specifically designed to meet the requirements of nuclear extracts used in various other epigenetic products from Epigentek, including DNMT, HDAC, and HAT assays. A total of 100 standard extractions (using 10^7 cells or 20 mg tissues) can be performed with this kit. Total yield can be up to 100 µg per optimal extraction, although results may somewhat vary depending on the cell or tissue type. The *EpiQuik*™ Nuclear Extraction Kit has an extremely fast procedure, which can be completed within 60 minutes.

PROTOCOL

Cell Pellet Preparation

For Monolayer or Adherent Cells

1. Grow cells to 70-80% confluency in 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 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*.)
3. Centrifuge the cells for 5 minutes at 1000 rpm and discard the supernatant.
4. Dilute **NE1** with distilled water at a 1:10 dilution (1X). Add **DTT Solution** and **PIC** to ice cold **diluted NE1 (1X)** at a 1:1000 dilution. Re-suspend cell pellet in 100 µl of **diluted NE1 (1X)** per 10^6 cells and transfer to a micro-centrifuge vial.
5. Incubate on ice for 10 minutes. Vortex vigorously for 10 seconds then centrifuge the preparation for 1 minute at 12,000 rpm in a desktop centrifuge (about 11000 G).
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 x 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. Dilute **NE1** with distilled water at a 1:10 dilution (1X). Add **DTT Solution** and **PIC** to ice cold **diluted NE1 (1X)** at a 1:1000 dilution. Re-suspend cell pellet in 100 µl of **diluted NE1 (1X)** per 10^6 cells and transfer to a micro-centrifuge vial.
4. Incubate on ice for 10 minutes. Vortex vigorously for 10 seconds then 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.)

For Tissue Samples

1. Weigh tissue and cut it into small pieces. Place the cut pieces in a clean homogenizer.
2. Dilute **NE1** with distilled water at a 1:10 dilution(1X). Per gram of tissue, add 5 ml of **diluted NE1 (1X)** containing 5 µl of **DTT** and homogenize tissue pieces (50-60 strokes).
3. Incubate on ice for 15 minutes then centrifuge for 10 minutes at 12,000 rpm at 4°C. Remove the supernatant.

Nuclear Extract Preparation

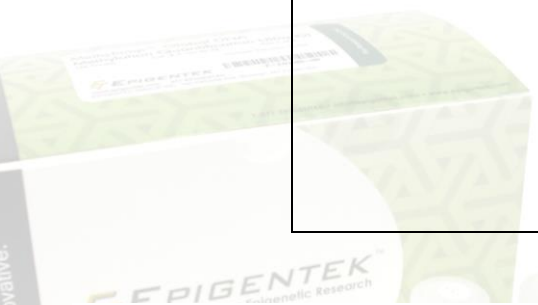
1. Add **DTT Solution*** and **PIC** to **NE2** at a 1:1000 dilution. Add 2 volumes (based on pellet size) of **NE2** containing **DTT** and **PIC** to nuclear pellet (about 10 µl per 10^6 cells or per 2 mg of tissue). Incubate the extract on ice for 15 minutes with vortex (5 seconds) every 3 minutes. 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 micro-centrifuge vial.
3. Measure the protein concentration of the nuclear extract.

   Note: A standard Bradford protein assay to measure the concentration of the nuclear extract is recommended. For accurate nuclear extract quantification, the final working buffer (NE2+PIC with or without DTT) should be used as a blank. After the protein concentration has been measured the DTT can be added for storage purposes.

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

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of nuclear proteins	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 **NE1** 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 **NE1** 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. Standard shelf life, when stored properly is 1 year from date of receipt.
	Incorrect temperature and/or insufficient incubation time during extraction.	Ensure the incubation time and temperatures described in the protocol are followed correctly.
Low/no activity of nuclear enzymes in downstream activity assays	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; 2) the blank and samples turned a dark purple color immediately upon adding nuclear extracts or final working buffer for blank	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.

RELATED PRODUCTS

OP-0022 EpiQuik™ Nuclear Extraction Kit II (Nucleic Acid-Free)