EpiQuik™ Nuclear Extraction Kit II

Base Catalog # OP-0022

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Nuclear Extraction Kit II (Nucleic Acid-Free) is suitable for fast preparation of nucleic acid-free nuclear proteins from mammalian cells and tissue samples.

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KIT CONTENTS

Components	100 extractions OP-0022-100
NP1 (10X Pre-Extraction Buffer)	10 ml
NP2 (Extraction Buffer)	10 ml
NP3 (Extraction Pre-Cleaner)	1 ml
NP4 (Extraction Cleaner)	100 µl
1000X DTT Solution	100 µl
1000X Protease Inhibitor Cocktail (PIC)	100 µl

SHIPPING & STORAGE

Upon receipt: (1) Store **NP3** and **NP4** at –20°C in aliquots; (2) Store **all other components** at 4°C. The kit is stable for up to 1 year from the date of shipment, when stored properly.

GENERAL PRODUCT INFORMATION

Usage Limitations: The *EpiQuik*™ Nuclear Extraction Kit II (Nucleic Acid-Free) is for research use only and is not intended for diagnostic or therapeutic applications.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

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

A BRIEF OVERVIEW

The EpiQuik™ Nuclear Extraction Kit II (Nucleic Acid-Free) is a complete set of optimized reagents to provide a simple and selective method for isolating nuclear proteins free of nucleic acids attached, to be used in 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 nucleic acid-free nuclear proteins but not enzymatic activity. The protocol is fast and easy-to-use, and also isolates very abundant yields of nuclear extract from mammalian cells or tissue samples.

PROTOCOL

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 cm^2 area (e.g., add 3 ml of PBS to a100 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 **NP1** with distilled water at a 1:10 ratio (ex: 1 ml of **NP1** + 9 ml of distilled water). Add **DTT Solution** and **PIC** to ice cold **diluted NP1 (1X)** at a 1:1000 ratio. Re-suspend cell pellet in 100 µl of **diluted NP1 (1X)** 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.

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. Dilute **NP1** with distilled water at a 1:10 ratio (ex: 1 ml of **NP1** + 9 ml of distilled water). Add **DTT Solution** and **PIC** to ice cold **diluted NP1(1X)** at a 1:1000 ratio. Re-suspend cell pellet in 100 µl of **diluted NP1 (1X)** 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.

For Tissue Samples:

1. Weigh the tissue and cut it into small pieces. Place tissue pieces in a clean homogenizer.
2. Dilute **NP1** with distilled water at a 1:10 ratio (ex: 1 ml of **NP1** + 9 ml of distilled water). Add 5 ml of **diluted NP1 (1X)** 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. Remove the supernatant.

Nuclear Extract Preparation

1. Add **DTT Solution** and **PIC** to **NP2** at a 1:1000 ratio, followed by adding **NP3** to **NP2** at a 1:10 ratio. Add 2 volumes of **NP2** to nuclear pellet (about 10 µl **NP2** 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. Add NP4 to the supernatant at a 1:100 ratio (ex: add 10 µl of NP4 to 990 µl of the supernatant and incubate for 15-20 minutes at room temperature.

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

5. Measure the protein concentration of the nuclear extract.

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

RELATED PRODUCTS

OP-0002-100	EpiQuik™ Nuclear Extraction Kit I