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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
User Guide	1

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.

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