EpiQuik™ Nuclear Extraction Kit I

Catalog No. OP-0002-1

INTRODUCTION

The EpiQuik™ Nuclear Extraction Kit provides the simple and selective method for extracting nuclear proteins to be used for a variety of applications. These applications may include western blotting, protein-DNA binding assays, nuclear enzyme assays, and any other procedures requiring optimization or nucleic acidfree nuclear proteins. The EpiQuik™ Nuclear Extraction Kits are also specifically designed to meet the requirements of nuclear extracts used in various EpiQuik™ assays. The EpiQuik™ Nuclear Extraction Kits can be used to extract nuclear proteins from mammalian cells and tissue samples. The EpiQuik™ Nuclear Extraction Kits include kit I for regular nuclear extraction and kit II for nucleic acid-free nuclear extraction. The EpiQuik™ Nuclear Extraction Kit has the fastest procedure available on the current market, which can be completed within 60 minutes.

PRODUCT USE INFORMATION

The EpiQuik™ Nuclear Extraction Kit is very suitable for quick preparation of nuclear extracts from mammalian cells and tissue samples.

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

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

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

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KIT CONTENTS

Components 100 extractions OP-0002-1

NE1 (10X pre-extraction buffer) 10 ml
NE2 (extraction buffer) 10 ml
1000X DTT solution 110 µl
1000X protease inhibitor cocktail (PIC) 110 µl

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

SHIPPING AND STORAGE

Store all components at 4°C. The kit is stable for up to 1 year from date of shipment when stored properly.

PROTOCOL

Cell Pellet Preparation

For Monolayer or Adherent Cells:
1. Grow cells to 70-80% confluence on a culture plate or flask (about 2-5 X 106 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. (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 min at 1000 rpm and discard the supernatant.

4. Dilute NE1 with distilled water at a 1:10 ratio. Add DTT solution and PIC to ice cold diluted NE1 (1X) at a 1:1000 ratio. Re-suspend cell pellet in 100 µl of diluted NE1 per 106 cells and transfer to a micro centrifuge vial.

5. Incubate on ice for 10 min. Vortex vigorously for 10 sec and centrifuge the preparation for 1 min at 12,000 rpm.

6. Carefully remove the cytoplasmic extract from the nuclear pellet.

For Suspension Cells:

1. Grow cells to 2 X 106/ml and collect the cells into a 15 ml conical tube.

2. Centrifuge the cells for 5 min at 1000 rpm and discard the supernatant. Wash cells with PBS once by centrifugation for 5 min at 1000 rpm. Discard the supernatant.

3. Dilute NE1 with distilled water at a 1:10 ratio. Add DTT solution and PIC to ice cold diluted NE1 (1X) at a 1:1000 ratio. Re-suspend cell pellet in 100 µl of diluted NE1 per 106 cells and transfer to a micro centrifuge vial.

4. Incubate on ice for 10 min. Vortex vigorously for 10 sec and centrifuge the preparation for 1 min at 12,000 rpm.

5. Carefully remove the cytoplasm extract from the nuclear pellet.

For Tissue Samples:

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

2. Dilute NE1 with distilled water at a 1:10 ratio. Add 5 ml of diluted NE1 (1X) containing 5 µl of DTT per gram of tissue and homogenize tissue pieces (50-60 strokes).

3. Incubate on ice for 15 min and centrifuge for 10 min 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 ratio. Add 2 volumes of NE2 containing DTT and PIC to nuclear pellet (about 10 µl per 106 cells or per 2 mg tissues). Incubate the extract on ice for 15 min with vortex (5 sec) every 3 min. The extract (especially tissue extract) can be further sonicated for 3 X 10 sec to increase nuclear protein extraction.

2. Centrifuge the suspension for 10 min 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.

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