Product Manual

EpiXtract™ Nuclear Protein Isolation Kit II (Nucleic acid-free)

Catalog #: ENZ-45015

100 Assays

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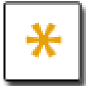

Please read entire booklet before proceeding with the assay.

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INTRODUCTION

The EpiXtract™ Nuclear Protein Isolation Kit II (nucleic acid-free) uses a selective and easy to use method for extracting nuclear proteins that can be used for a variety of downstream applications. Applications include Western blot analysis, protein-DNA binding assays, nuclear enzyme assays, and any other procedures that require optimization or the use isolated nucleic acid-free nuclear proteins. The EpiXtract™ Nuclear Protein Isolation Kit II (nucleic acid-free) is compatible with mammalian adherent or suspension cells and tissue samples.

Using the EpiXtract™ Nuclear Protein Isolation Kit II, the extraction procedure can be completed within 1 hour. Under optimal extraction conditions a total yield can be up to 100µg.

SAFETY WARNINGS & PRECAUTIONS

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Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

REAGENTS PROVIDED AND STORAGE

The EpiXtract™ Nuclear Protein Isolation Kit II is shipped on ice.

Upon receipt: (1) Store **Extraction Pre-Cleaner** and **Extraction Cleaner** store at –20°C in aliquots; and (2) Store all other components at 4°C.

Reagent	Volume	Storage Temperature
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	100 µL	4°C

ADDITIONAL MATERIALS REQUIRED

- Adjustable pipette
- Aerosol resistant pipette tips
- Distilled water
- Conical tubes
- microcentrifuge vials
- tissue homogenizer
- Vortex
- Microcentrifuge
- Hemacytometer
- Freshly made PBS solution
- UV Spectrometer
- Ice

METHODS AND PROCEDURES

Monolayer or adherent cells preparation

1. Grow cells to 70-80% confluency on a culture plate or flask. Cell density should not exceed 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 1mL 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. Alternatively, cells can be detached with trypsin/EDTA, collected into a 15 mL conical tube and counted with a hemacytometer.
3. Centrifuge the cells for 5 min at 1000 rpm and discard the supernatant.
4. Dilute the **10x Pre-Extraction Buffer** with distilled water at a 1/10 ratio (e.g., 1 mL of **10x Pre-Extraction Buffer** and 9 mL of distilled water) Add **1000x DTT Solution** and **1000x Protease Inhibitor Cocktail** to ice cold diluted **(1x) Pre-Extraction Buffer** at a 1/1000 ratio. Resuspend cell pellet in 100 µL of this mix per 10^6 cells. Transfer to a microcentrifuge vial.
5. Incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge the preparation for 1 minute at 12,000rpm.
6. Carefully remove the cytoplasmic extract from the nuclear pellet.
7. Proceed to **Section D, Nuclear Extraction.**

Suspension cells preparation

1. Grow cells to a 2 x 10^6/ml. Collect the cells in a 15 mL conical tube.
2. Centrifuge the cells for 5 minutes at 1000rpm and discard the supernatant. Wash cells with PBS once by centrifugation for 5 minutes at 1000rpm. Discard the supernatant.
3. Dilute the **10x Pre-Extraction Buffer** with distilled water at a 1/10 ratio (e.g., 1 mL of **10x Pre-Extraction Buffer** and 9mL of distilled water). Add **1000x DTT Solution** and **1000x Protease Inhibitor Cocktail** to ice cold diluted **(1x) Pre-Extraction Buffer** at a 1/1000 ratio. Resuspend cell pellet in 100µL of this mix per 10^6 cells. 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 12,000rpm.
5. Carefully remove the cytoplasmic extract from the nuclear pellet.
6. Proceed to **Section D, Nuclear Extraction.**

Tissue sample preparation

1. Weigh tissue and cut it into small pieces. Place the cut pieces in a clean homogenizer
2. Dilute the **10x Pre-Extraction Buffer** with distilled water at a 1/10 ratio (e.g., 1 mL of **10x Pre-Extraction Buffer** and 9mL of distilled water). Add 5 mL of diluted (1x) **Pre-Extraction Buffer** 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 12,000rpm at 4°C. Remove the supernatant.
4. Proceed to **Section D, Nuclear Extraction.**

Nuclear extraction

1. Add **DTT Solution** and **Protease Inhibitor Cocktail** to **Extraction Buffer** at a 1/1000 ratio, followed by adding **Extraction Pre-Cleaner** to **Extraction Buffer** at a 1/10 ratio. Add 2 volumes of **Extraction Buffer** mix to the nuclear pellet (about 10µL **Extraction Buffer** mix per 10^6 cells or per 2 mg of tissue).
2. Incubate the extract on ice for 15 minutes. Vortex (for 5 seconds) every 3 minutes. The extract (especially tissue extract) can be further sonicated for 3 x 10 seconds to increase nuclear protein extraction.
3. Centrifuge the suspension for 10 minutes at 14,000rpm at 4°C. Transfer the supernatant into a new microcentrifuge vial.
4. Add **Extraction Cleaner** to the supernatant at a 1/100 ratio (e.g., add 10 µL of **Extraction Cleaner** to 990 µL of the supernatant) and incubate for 15-20 minutes at room temperature.
5. Centrifuge the suspension for 1 minute at 14,000rpm at 4°C and transfer the supernatant into a new microcentrifuge vial.
6. Measure the protein concentration of the nuclear extract.
7. Use immediately or aliquot and freeze the supernatant at -80°C until further use. Avoid freeze/thaw cycle.

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GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com