

ReadiPrep™ Nuclear/Cytoplasmic Fractionation Kit

Catalog number: 60000

Unit size: 50 Tests

Component	Storage	Amount
Component A: Cytosol Extraction Buffer	Refrigerate (2-8 °C)	1 Bottle (25 mL)
Component B: Nuclear Extraction Buffer	Refrigerate (2-8 °C)	1 Bottle (8 mL)
Component C: 10X Protease Inhibitors	Freeze (<-15 °C)	1 Vial (4 mL)
Component D: DTT (1 M)	Freeze (<-15 °C)	1 Vial (100 µL)

OVERVIEW

The ReadiPrep™ Nuclear/Cytoplasmic Fractionation Kit offers stepwise isolation of nuclear and cytoplasmic extract from mammalian cells or tissue. The isolated proteins have high concentrations and preserve their biological activities. The nuclear and cytoplasmic extracts from ReadiPrep™ Nuclear/Cytoplasmic Fractionation Kit are compatible with many downstream applications including enzyme activity assays and fluorescent Western blotting etc.

AT A GLANCE

Protocol summary

1. Rinse cells with PBS
2. Add 500 µL of Cytosol Extraction Buffer
3. Centrifuge for 20 seconds
4. Collect the supernatant (Cytoplasmic extract)
5. Re-suspend the pellets in 150 µL 1X High Salt Buffer
6. Centrifuge for 20 minutes
7. Collect the supernatant (Nuclear Extract)

Important Thaw all the kit components at room temperature before starting the experiment (you may store buffers at 4°C or -20°C), keep the buffers on ice during the experiment.

PREPARATION OF WORKING SOLUTION

1. Cytosol Extraction Buffer (1X):

Add 100 µL of 10X Protease Inhibitors (Component C) and 1 µL of DTT (Component D) into 0.9 mL of Cytosol Extraction Buffer (Component A).

2. Nuclear Extraction Buffer (1X):

Add 100 µL of 10X Protease Inhibitors (Component C) and 1 µL of DTT (Component D) into 0.9 mL of Nuclear Extraction Buffer (Component B).

Note 0.5 mL 1X Cytosol Extraction Buffer and 150 µL of 1X Nuclear Extraction Buffer is enough for 1 assay, prepare fresh buffer as needed.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit

<https://www.aatbio.com/resources/guides/cell-sample-preparation.html>

SAMPLE EXPERIMENTAL PROTOCOL

1. Rinse the cells 1 time with cold PBS. For cells in suspension, collect the cells by centrifugation.
2. Add 500 µL of 1X Cytosol Extraction Buffer to cells. For adherent cells, scrape the adherent cells into a 1.5 mL centrifuge tube.
3. Vortex vigorously to fully re-suspend the cells.
4. Centrifuge at 16,000 g for 1 - 2 minutes and transfer the supernatant

(Cytoplasmic extract) to another clean tube. Keep the tube on ice for downstream applications or store at -80°C.

5. Re-suspend the pellet in 150 µL 1X Nuclear Extraction Buffer.
6. Vortex vigorously to fully re-suspend the pellet.
7. Rotate the tube at 4°C for 30 minutes.
8. Centrifuge at 16,000g for 20 minutes and transfer the supernatant (Nuclear extract) to another clean tube. Keep the tube on ice for downstream applications or store at -80°C.

EXAMPLE DATA ANALYSIS AND FIGURES

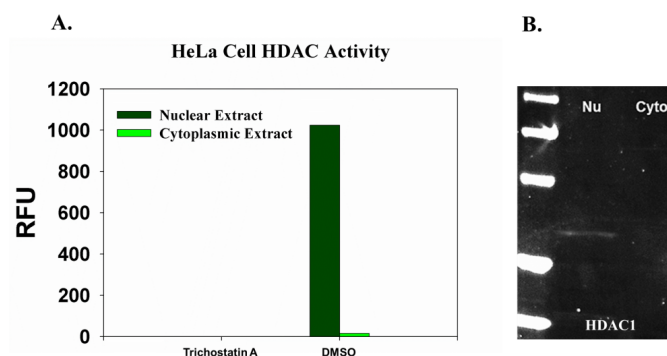


Figure 1. Nuclear and cytoplasmic extract from HeLa cells were collected using ReadiPrep™ Nucleus/Cytosol Fractionation Kit (AAT Bioquest®, Cat#60000). The protein was quantified by Amplitude™ Fluorimetric Fluorescamine Protein Quantitation Kit (AAT Bioquest®, Cat#. 11100). **A.** 8 µg of nuclear or cytoplasmic extract was incubated with or without HDAC inhibitor Trichostatin A, and HDAC activity was measured by Amplitude™ Fluorimetric HDAC Activity Assay Kit (AAT Bioquest®, Cat#. 13601). **B.** 40 µg total protein of nuclear or cytoplasmic extract was used. 2 µg/ml of rabbit anti-HDAC1 antibody was used to probe the nitrocellulose membrane for overnight. 10 µg/ml of iFluor™ 647 goat anti-rabbit IgG (H+L) (AAT Bioquest®, Cat#. 16809) was used. The detection was visualized by UVP MultiSpectral Imaging System (Biolite). M: Marker, Nu: Nuclear extract, Cyto: Cytoplasmic extract.

DISCLAIMER

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