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Product Manual

# Nuclear/Cytosolic Fractionation Kit

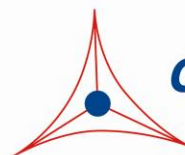
Catalog Number

AKR-171

20 preps

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **Introduction**

Cell Biolabs' Nuclear/Cytosolic Fractionation Kit provides a simple and fast tool to isolate nuclear extract from the cytoplasmic fraction of mammalian cells. The procedure has been optimized to provide extraction, with high protein recovery and low cross-contamination, in less than 2 hours. The extracted protein fractions are functional and suitable for downstream assays such as DNA footprinting, RNA splicing, gel shift assays (EMSA), reporter assays, enzyme activity assays, and Western blotting. Each kit provides sufficient quantities to perform 20 preps (up to  $5 \times 10^6$  cells each).

## **Related Products**

1. AKR-103: PhosphoBlocker™ Blocking Reagent (1L)
2. AKR-172: Nuclear/Cytosolic Fractionation Kit (100 Preps)

## **Kit Components**

1. Cytosol Extraction Buffer, Hypotonic (10X) (Part No 217101): One 2 mL vial.
2. Cell Lysis Reagent (Part No. 217102): One 1 mL vial of 10% Igepal CA-630 in 1X Cytosol Extraction Buffer (CEB).
3. Nuclear Extraction Buffer (Part No. 217103): One 2 mL vial.
4. Dithiothreitol (1000X) (Part No. 217104): One 20 µL vial of 1 M DTT.
5. Protease Inhibitor Cocktail (100X) (Part No. 217105): One 200 µL vial containing AEBSF, Aprotinin, Bestatin, E64, Leupeptin, and Pepstatin A in DMSO.

## **Materials Not Supplied**

1. PBS
2. Microcentrifuge tubes
3. Microcentrifuge

## **Storage**

Upon receiving, aliquot and store Dithiothreitol and Protease Inhibitor Cocktail at  $-20^{\circ}\text{C}$  and avoid multiple freeze/thaw cycles. Store all other components at  $4^{\circ}\text{C}$ .

## **Preparation of Reagents**

- 1X Cytosol Extraction Buffer (CEB): Dilute the 10X Cytosol Extraction Buffer to 1X with deionized water. Stir to homogeneity.
- Dithiothreitol: Immediately before use dilute the Dithiothreitol 1:1000 with 1X Cytosol or Nuclear Extraction Buffer. Stir to homogeneity. Do not store diluted solutions.
- Protease Inhibitor Cocktail: Immediately before use dilute the Protease Inhibitor Cocktail 1:100 with 1X Cytosol or Nuclear Extraction Buffer. Stir to homogeneity. Do not store diluted solutions.

## **Preparation of Samples**

### **I. Adherent Cells**

1. Culture cells to approximately 80-90% confluence.
2. Aspirate the culture media and wash twice with PBS.
3. Detach the cells from the plates in PBS by scraping with a cell scraper.
4. Collect the solution into an appropriate conical centrifuge tube.
5. Centrifuge for 5 minutes (600 x g).
6. Discard the supernatant and immediately proceed to the Assay Protocol.

### **II. Suspension Cells**

1. Collect the cells into an appropriate conical centrifuge tube.
2. Centrifuge for 5 minutes (600 x g).
3. Remove and discard the supernatant.
4. Wash the cells twice with PBS.
5. Centrifuge for 5 minutes at (600 x g).
6. Discard the supernatant and immediately proceed to the Assay Protocol.

## **Assay Protocol**

*Important Note: Perform the below steps at 2-8°C. All buffers, centrifuge rotors, and equipment should be maintained at 2-8°C. Before use, Dithiothreitol and Protease Inhibitor Cocktail should be diluted according to the Preparation of Reagents section above.*

### **I. Cytosol Fractionation Protocol**

1. Collect cells (up to  $5 \times 10^6$ ) by centrifugation for 5 minutes at 4°C (600 x g).
2. Wash the cells once with ice cold PBS.
3. Remove and discard the supernatant.
4. Gently resuspend the cell pellet with 500 µL of ice cold, 1X Cytosol Extraction Buffer (containing DTT/Protease Inhibitors) by pipetting up and down.
5. Transfer the suspension into a prechilled microcentrifuge tube.
6. Incubate on ice for 10 minutes.
7. Add 25 µL of Cell Lysis Reagent and vortex for 10 seconds at the highest setting.
8. Centrifuge for 10 minutes at 4°C (800 x g).
9. Carefully transfer the supernatant (cytoplasmic fraction) to a clean, chilled microcentrifuge tube. The cytoplasmic fraction can be stored at -80°C for future use.

*Note: Make sure not to disturb/remove the nuclei pellet.*

10. Gently resuspend the pellet with 500  $\mu$ L of ice cold, 1X Cytosol Extraction Buffer (containing DTT/Protease Inhibitors) by pipetting up and down.

*Note: This wash step is included to reduce cross-contamination between fractions.*

11. Add 25  $\mu$ L of Cell Lysis Reagent and vortex for 10 seconds at the highest setting.

12. Centrifuge for 10 minutes at 4°C (800 x g).

13. Carefully aspirate the supernatant and discard of this wash.

## II. Nuclear Protein Extraction Protocol

1. Gently resuspend the nuclear pellet with 100  $\mu$ L of ice cold, 1X Nuclear Extraction Buffer (containing DTT/Protease Inhibitors) by pipetting up and down.

2. Maintain on ice for 30 minutes, vortexing for 10 seconds at the highest setting in 10 minute intervals.

3. Centrifuge for 30 minutes at 4°C (14000 x g).

4. Carefully transfer the supernatant (nuclear protein extract) to a clean, chilled microcentrifuge tube. The extract can be stored at -80°C for future use.

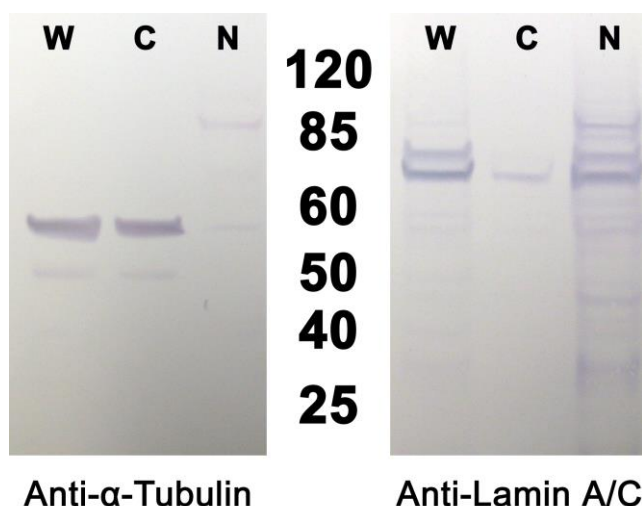
*Note: The nuclear extract typically yields protein concentrations of > 1 mg/mL. If greater concentrations are desired, resuspend the nuclear pellet in a smaller volume in step 1 above (minimum of 25  $\mu$ L).*

## III. Other Considerations

- For determining the protein content of extracts, samples must be diluted 1:2 before running in the Bradford Protein Assay. Buffer only controls must be performed concurrently. DTT in the buffers is not compatible with the BCA Protein Assay.
- Nuclear Extraction Buffer is a high salt buffer, containing 420 mM NaCl. If salt removal is necessary, dialysis or a desalting column may be used.

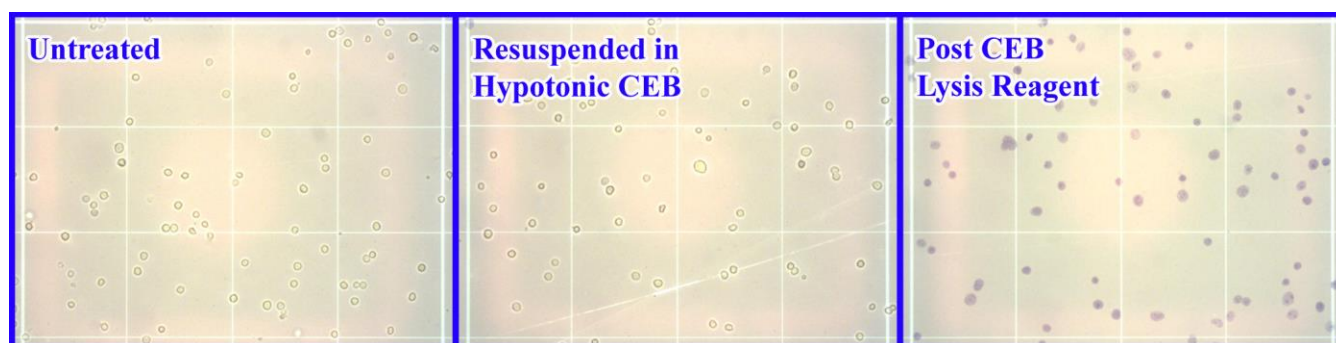
## Example of Results

The following figure demonstrates typical results seen with Cell Biolabs' Nuclear/Cytosolic Fractionation Kit. One should use the data below for reference only.



**Figure 1: HEK293 Cell Fractionation.** Cytosolic and nuclear protein extracts were isolated from Human Embryonic Kidney 293 cells according to the Assay Protocol. Whole cell (W), cytosol (C), and nuclear (N) fractions were immunoblotted with Anti- $\alpha$ -Tubulin (left) or Anti-Lamin A/C (right) at 1  $\mu$ g/mL.

*Note: Anti- $\alpha$ -Tubulin (Calbiochem CP06) and Anti-Lamin A/C (Sigma SAB4200236) are both mouse monoclonals. Tubulin and Lamin are known to be cytosolic and nuclear specific proteins, respectively.*



**Figure 2: HEK293 Trypan Blue Staining.** Human Embryonic Kidney 293 cells were stained with Trypan Blue at various steps during the fractionation protocol, demonstrating complete lysis and high nuclei recovery.

## Recent Product Citations

1. Geng, C. & Ou, S. (2021). Higenamine Induces Glioma Cell Death by Modulating Nuclear Factor- $\kappa$ B Nuclear Translocation, Phosphoinositide-3-Kinase/Protein Kinase B Signaling and Caspase Cascade. *Curr Top Nutraceutical Res.* **19**(3):317-325. doi: 10.37290/ctrn2641-452X.19:317-325.

2. Li, J. et al. (2021). LncRNA NOP14-AS1 Promotes Tongue Squamous Cell Carcinoma Progression by Targeting MicroRNA-665/HMGB3 Axis. *Cancer Manag Res.* **13**:2821-2834. doi: 10.2147/CMAR.S293322.
3. Bhatt, A.B. et al. (2021). Diverse and converging roles of ERK1/2 and ERK5 pathways on mesenchymal to epithelial transition in breast cancer. *Transl Oncol.* **14**(6):101046. doi: 10.1016/j.tranon.2021.101046.
4. Ushimaru, S. et al. (2020). Roles of Layilin in Regulation of Low-Density Lipoprotein Receptor in Malignant Glioma Cells. *J. St. Marianna Univ.* **11**:53-59.
5. Kumar, A. et al (2020). Actin R256 Mono-methylation Is a Conserved Post-translational Modification Involved in Transcription. *Cell Rep.* **32**(13):108172. doi: 10.1016/j.celrep.2020.108172.
6. Hwang, S.G. et al. (2020). Cold atmospheric plasma prevents wrinkle formation via an anti-aging process. *Plasma Med.* doi: 10.1615/PlasmaMed.2020034810.
7. Sadek, J. et al. (2020). Modulation of virus-induced NF- $\kappa$ B signaling by NEMO coiled coil mimics. *Nat Commun.* **11**(1):1786. doi: 10.1038/s41467-020-15576-3.
8. Xiao, G. et al. (2019). Bacoside A attenuates nephrotoxicity and acute kidney injury in male albino rats induced by cisplatin. *Int. J. Pharmacol.* **15**:257-264. doi: 10.3923/ijp.2019.257.264.
9. Zeng, X. et al. (2019). Adenovirus early region 3 RIDa protein limits NF $\kappa$ B signaling through stress-activated EGF receptors. *PLoS Pathog.* **15**(8):e1008017. doi: 10.1371/journal.ppat.1008017.
10. Okuno, Y. et al. (2019). Bioactivation Mechanisms of N-hydroxyaristolactams: Nitroreduction Metabolites of Aristolochic Acids. *Environ Mol Mutagen.* doi: 10.1002/em.22321.
11. Hao, Y. et al. (2019). Cardioprotective Efficacy of Naringenin Against Isoproterenol Induced Chronic Heart Failure in a Rat Model. *International Journal of Pharmacology.* **15**: 759-765. doi: 10.3923/ijp.2019.759.765.
12. Kaji, T. et al. (2019). Layilin enhances the invasive ability of malignant glioma cells via SNAI1 signaling. *Brain Res.* pii: S0006-8993(19)30289-6. doi: 10.1016/j.brainres.2019.05.034.
13. Lin, X. et al. (2019). Curcumin attenuates oxidative stress in RAW264.7 cells by increasing the activity of antioxidant enzymes and activating the Nrf2-Keap1 pathway. *PLoS One.* **14**(5):e0216711. doi: 10.1371/journal.pone.0216711.
14. Sun, S. et al. (2019). Icaritin Attenuates High Glucose-Induced Apoptosis, Oxidative Stress, and Inflammation in Human Umbilical Venous Endothelial Cells. *Planta Med.* **85**(6):473-482. doi: 10.1055/a-0837-0975.
15. Xiao, G. et al. (2019). Bacoside a Attenuates Nephrotoxicity and Acute Kidney Injury in Male Albino Rats Induced by Cisplatin. *International Journal of Pharmacology.* **15**: 257-264. doi: 10.3923/ijp.2019.257.264.
16. Hu, W. et al. (2019). Circular RNA circRNA\_15698 aggravates the extracellular matrix of diabetic nephropathy mesangial cells via miR-185/TGF- $\beta$ 1. *J Cell Physiol.* **234**(2):1469-1476. doi: 10.1002/jcp.26959.
17. Kim, G. et al. (2018). Combined delivery of curcumin and the heme oxygenase-1 gene using cholesterol-conjugated polyamidoamine for anti-inflammatory therapy in acute lung injury. *Phytomedicine.* **56**:165-174. doi: 10.1016/j.phymed.2018.09.240.
18. Yu, L. et al. (2018). LncRNA cancer susceptibility candidate 15 accelerates the breast cancer cells progression via miR-153-3p/KLF5 positive feedback loop. *Biochem Biophys Res Commun.* **506**(4):819-825. doi: 10.1016/j.bbrc.2018.10.131.

19. Liu, J. et al. (2018). Asiatic Acid Enhances Antioxidant and Anti-inflammatory Activity to Suppress Isoproterenol Induced Cardiotoxicity. *International Journal of Pharmacology*. **14**:1038-1045.
20. Yang, Y. et al. (2018). Nuclear GSK3 $\beta$  induces DNA double-strand break repair by phosphorylating 53BP1 in glioblastoma. *Int J Oncol*. **52**(3):709-720. doi: 10.3892/ijo.2018.4237.
21. Byeon, H.E. et al. (2017). MicroRNA-132 Negatively Regulates Palmitate-Induced NLRP3 Inflammasome Activation through FOXO3 Down-Regulation in THP-1 Cells. *Nutrients*. **9**(12). pii: E1370. doi: 10.3390/nu9121370.
22. Hou, W.C. et al. (2017). Withaferin A induces apoptosis in rat C6 glioma cells through regulating NF-KB nuclear translocation and activation of caspase cascade. *Afr J Tradit Complement Altern Med*. **14**(2):319-324. doi: 10.21010/ajtcam.v14i2.33.
23. Sun, Y. et al. (2017). Beneficial effect of 20-hydroxyecdysone exerted by modulating antioxidants and inflammatory cytokine levels in collagen-induced arthritis: A model for rheumatoid arthritis. *Mol Med Rep*. **16**(5):6162-6169. doi: 10.3892/mmr.2017.7389.
24. Abe, Y. et al. (2017). Deep Phospho- and Phosphotyrosine Proteomics Identified Active Kinases and Phosphorylation Networks in Colorectal Cancer Cell Lines Resistant to Cetuximab. *Sci Rep*. **7**(1):10463. doi: 10.1038/s41598-017-10478-9.
25. Zhang, F. et al. (2017). Daidzein ameliorates spinal cord ischemia/reperfusion injury-induced neurological function deficits in Sprague-Dawley rats through PI3K/Akt signaling pathway. *Exp Ther Med*. **14**(5):4878-4886. doi: 10.3892/etm.2017.5166.
26. Fu, Z. et al. (2017). LncRNA HOTTIP modulates cancer stem cell properties in human pancreatic cancer by regulating HOXA9. *Cancer Lett*. **410**:68-81. doi: 10.1016/j.canlet.2017.09.019.
27. Alam, M.B. et al. (2017). DNA Protecting Activities of Nymphaea nouchali (Burm. f) Flower Extract Attenuate t-BHP-Induced Oxidative Stress Cell Death through Nrf2-Mediated Induction of Heme Oxygenase-1 Expression by Activating MAP-Kinases. *Int J Mol Sci*. **18**(10). pii: E2069. doi: 10.3390/ijms18102069.
28. Mei, X. et al. (2017). Neuroprotective effects of  $\alpha$ -lipoic acid against hypoxic-ischemic brain injury in neonatal rats. *Trop. J. Pharm. Res*. **16**(5):1051-1058.
29. Rahman, M.S. et al. (2017). A novel antioxidant peptide, purified from *Bacillus amyloliquefaciens*, showed strong antioxidant potential via Nrf-2 mediated heme oxygenase-1 expression. *Food Chem*. **239**:502-510.
30. Yamazaki, H. et al. (2017). Hypoxia-activated prodrug TH-302 decreased survival rate of canine lymphoma cells under hypoxic condition. *PLoS One* **12**(5):e0177305.

## **Warranty**

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## **Contact Information**

Cell Biolabs, Inc.  
7758 Arjons Drive  
San Diego, CA 92126  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

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