

Fractionate subcellular proteins for localization studies

Segregate and enrich proteins from five cellular compartments

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Fractionation of subcellular proteins enables protein localization assessment and protein enrichment from specific cellular compartments. The Thermo Scientific Subcellular Protein Fractionation Kit includes a combination of reagents for stepwise lysis of cells into functional cytoplasmic, membrane, soluble nuclear, chromatin-bound and cytoskeletal protein fractions in less than three hours.

Highlights:

- **Convenient** – perform a simple procedure without using ultracentrifugation over gradients
- **Complete** – obtain cytoplasmic, membrane, soluble nuclear, chromatin-bound and cytoskeletal protein fractions from a single kit
- **Compatible** – use extracts for downstream applications such as protein assays, Western blotting, gel-shift assays and enzyme activity assays

The Subcellular Protein Fractionation Kit contains four extraction buffers, a stabilized nuclease and Thermo Scientific Halt Protease Inhibitor Cocktail. Each kit includes reagents to fractionate 50 cell pellets of 20 μ l, equivalent to approximately 2 g of cell paste or tissue. The first reagent added to a cell pellet causes selective membrane permeabilization, releasing soluble cytoplasmic contents. The second reagent dissolves plasma, mitochondria and ER/golgi membranes but does not solubilize the nuclear membranes. After recovering intact nuclei by centrifugation, a third reagent yields the soluble nuclear extract. An additional nuclear extraction with micrococcal nuclease is performed to release chromatin-bound nuclear proteins. The recovered insoluble pellet is then extracted with the final reagent to isolate cytoskeletal proteins (Figure 1).

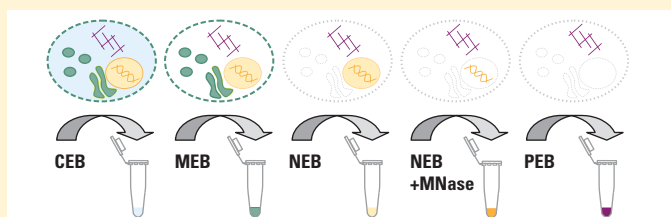


Figure 1. Schematic of the subcellular fractionation procedure. Cellular compartments are sequentially extracted by incubating cells with cytoplasmic extraction buffer (CEB) followed by membrane extraction buffer (MEB) and nuclear extraction buffer (NEB). Adding micrococcal nuclease to NEB extracts chromatin-bound proteins from the cell pellet before adding the pellet extraction buffer (PEB) to solubilize cytoskeletal proteins.

Extracts obtained with the Subcellular Protein Fractionation Kit are compatible with a variety of downstream applications and products including Western blotting, the Thermo Scientific Pierce BCA Protein Assay (Product # 23225), the Thermo Scientific LightShift Chemiluminescent EMSA Kit (Product # 20148), and reporter-gene and enzyme-activity assays. Extracts from each cellular compartment generally have less than 15% contamination between fractions, which is sufficient purity for most experiments studying protein localization and redistribution.

Applications:

To demonstrate the Subcellular Protein Fractionation Kit for studying protein localization, protein extracts were prepared from HeLa cells. Western blots were performed using specific antibodies against protein markers for various cellular compartments. Markers for cytoplasmic, membrane, soluble nuclear, chromatin-bound and cytoskeletal proteins were separated into distinct fractions with minimal cross-contamination (Figure 2). All protein fractions are also compatible with isoelectric focusing for 2D electrophoresis after minimal sample preparation using the Thermo Scientific 2D Sample Preparation for Soluble Proteins (Product # 89865) (data not shown).

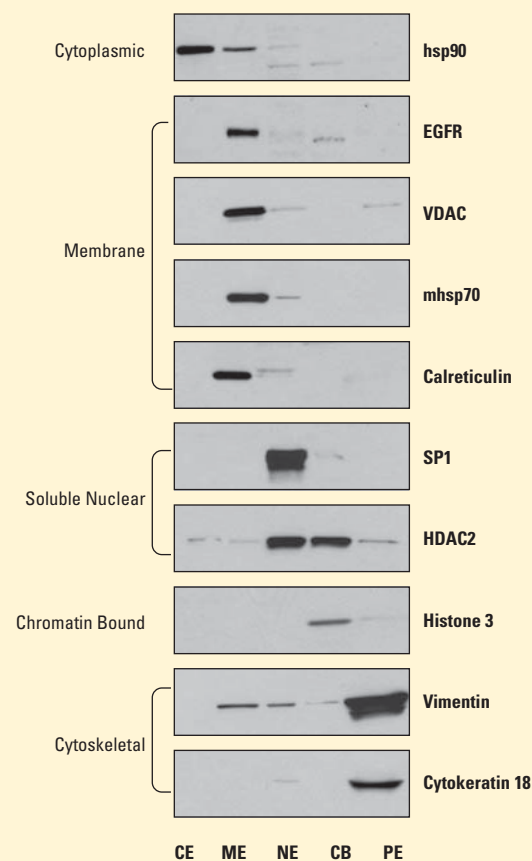
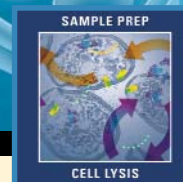


Figure 2. Western blots of fractionated cellular proteins. HeLa cells (2×10^6) were fractionated using the Subcellular Protein Fractionation Kit. Each extract (10 μ g) was analyzed by four Western blots using specific antibodies against proteins from various cellular compartments including cytoplasmic (hsp90), plasma membrane (EGFR), mitochondria membrane (VDAC), soluble mitochondria (mhsp70), endoplasmic reticulum (calreticulin), soluble nuclear (SP1 and HDAC2); chromatin-bound (histone 3); and cytoskeleton (cytokeratin 18 and vimentin). Goat anti-rabbit (H+L) HRP or goat anti-mouse (H+L) HRP was used as the secondary antibody, and Thermo Scientific SuperSignal West Dura Chemiluminescent Substrate (Product # 34076) was used for detection. **CE:** cytoplasmic extract, **ME:** membrane extract, **NE:** nuclear extract, **CB:** chromatin-bound extract, **PE:** pellet extract.



Although protein translocation typically occurs after proteins are synthesized, post-translational translocation events are highly indicative of protein signaling. NF κ B is a transcription factor involved in apoptotic signaling that is present in the cytosol as an inactive complex. Active NF κ B moves into the nuclear compartment after stimulation of A549 cells with the cell-death ligand, TNF α (Figure 3A). Protein kinase C alpha (PKC α) is activated by diacylglycerol at the plasma membrane. After stimulation of serum-starved HeLa cells with the phorbol ester PMA, PKC α rapidly moves from the cytoplasmic fraction to the membrane fraction (Figure 3B). As with the Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Kit, soluble nuclear extracts generated using the Subcellular Fractionation Protein Kit are compatible with gel-shift assays to further characterize transcription factor activation states (Figure 4).

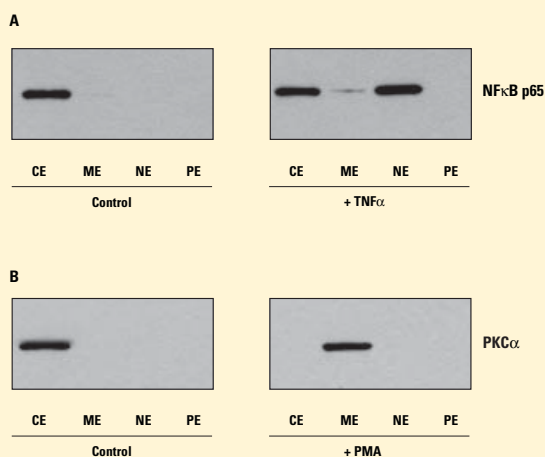


Figure 3. Western blots of subcellular fractionation to study protein translocation. Panel A: A549 cells (2×10^6) were either mock-treated or incubated with 20 μ g/ml TNF α for 20 minutes and fractionated using the Subcellular Protein Fractionation Kit. Each extract (10 μ g) was analyzed by Western blot using an anti-NF κ B p65 antibody. **Panel B:** Serum-starved HeLa cells (2×10^6) were either mock-treated or incubated with 1 μ M PMA for 20 minutes and fractionated. Each extract (10 μ g) was analyzed by Western blot using an anti-PKC α antibody. Goat anti-rabbit or anti-mouse (H+L) HRP was used as the secondary antibody, and SuperSignal[®] West Dura Chemiluminescent Substrate[†] (Product # 34076) was used for detection.

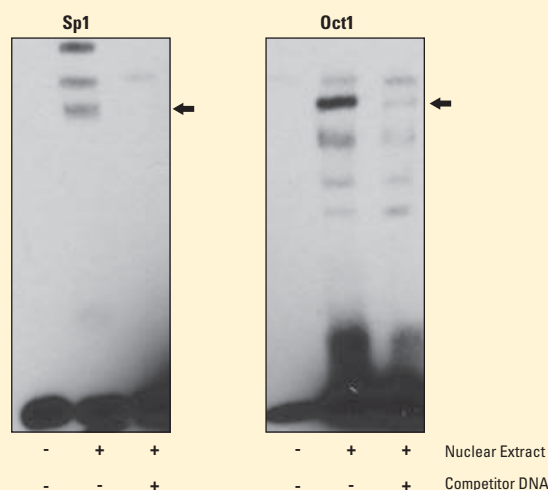


Figure 4. Electrophoretic mobility shift assay. DNA binding reactions were performed using 20 fmol biotin-labeled DNA duplex and 20 μ g nuclear extract prepared from HeLa cells using the Subcellular Protein Fractionation Kit. A 200-fold molar excess of unlabeled specific duplex was used for reactions containing specific competitor DNA. The Thermo Scientific LightShift Chemiluminescent EMSA Kit (Product # 20148) was used for detection.

Ordering Information:

Product #	Description	Pkg. Size
78840	Subcellular Protein Fractionation Kit Sufficient reagent for 50 cell preps. Includes: Cytoplasmic Extraction Buffer (CEB) 10 ml Membrane Extraction Buffer (MEB) 10 ml Nuclear Extraction Buffer (NEB) 10 ml Pellet Extraction Buffer (PEB) 5 ml Micrococcal Nuclease 100 units/ μ l, 150 μ l Calcium Chloride (CaCl ₂) 100 mM, 250 μ l Halt Protease Inhibitor Cocktail 100X, 350 μ l	Kit
88216	Micrococcal Nuclease	100 units/ μ l, 150 μ l

* Trademark, see Trademark Index on page 19.

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