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Sequential fractionation and isolation of subcellular proteins from tissue or cultured cells

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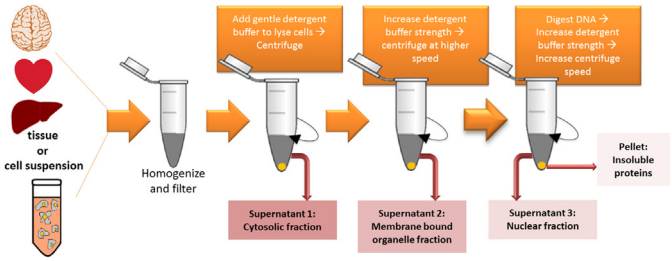
GRAPHICAL ABSTRACT



ABSTRACT

Many types of studies require the localization of a protein to, or isolation of enriched protein from a specific cellular compartment. Many protocols in the literature and from commercially available kits claim to yield pure cellular fractions. However, in our hands, the former often do not work effectively and the latter may be prohibitively expensive if a large number of fractionations are required. Furthermore, the largely proprietary composition of reagents in commercial kits means that the user is not able to make adjustments if, for example, a particular component affects the activity of a protein of interest. The method described here allows the isolation of purified proteins from three cellular fractions: the cytosol, membrane-bound organelles, and the nucleus. It uses gentle buffers with increasing detergent strength that sequentially lyse the cell membrane, organelle membranes and finally the nuclear membrane.

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- Quick, simple to replicate or adjust; this method does not require expensive reagents or use of commercial kits
- The protocol can be applied to tissue samples or cultured cells without changing buffer components
- Yields purified fractions of cytosolic, membrane bound and nuclear proteins, with the proper distribution of the appropriate subcellular markers: GAPDH, VDAC, SERCA2 and lamin A/C

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Method details

This method modifies a previously published protocol based on cultured cells [1], expanding it to allow for the collection of subcellular fractions from fresh tissue. In addition, the buffer compositions (Table 1) were optimized to minimize nuclear protein loss via the addition of 1 M hexylene glycol, which helps to further stabilize the membranes, especially that of the nucleus, and has been previously shown to yield highly enriched nuclear fractions [2]. HEPES is an organic buffer that stabilizes the pH of the solution while NaCl maintains the ionic strength [2].

The basis of this method (Fig. 1) is the sequential lysis of cell membranes by increasing the detergent strength of lysis buffers to obtain proteins from each fraction. Lysis buffer A is meant to release cytosolic proteins and its main component is digitonin. Digitonin is a steroidal saponin that permeabilizes the plasma membrane by binding with cholesterol and other β-hydroxysterols, thereby leading to the formation of pores in the membrane and its subsequent disruption. The advantage of

Table 1
Buffers required for the protocol.[a]

Lysis buffer A	
NaCl	150 mM
HEPES (pH 7.4)	50 mM
Digitonin (Sigma, D141)	25 μg/mL
Hexylene glycol (Sigma, 112100)	1 M
Protease inhibitor cocktail[b]	1% v:v
Lysis buffer B	
NaCl	150 mM
HEPES (pH 7.4)	50 mM
Igepal (Sigma, I7771)	1% v:v
Hexylene glycol	1 M
Protease inhibitor cocktail[b]	1% v:v
Lysis buffer C	
NaCl	150 mM
HEPES (pH 7.4)	50 mM
Sodium deoxycholate	0.5% w:v
Sodium dodecyl sulfate	0.1% w:v
Hexylene glycol	1 M
Protease inhibitor cocktail[b]	1% v:v

[a] Buffers (without protease inhibitor cocktail) can be stored at 4 °C for at least one month.
[b] Protease inhibitor cocktail (Sigma, P8340) should be added fresh at 1:100 dilution just prior to buffer use.