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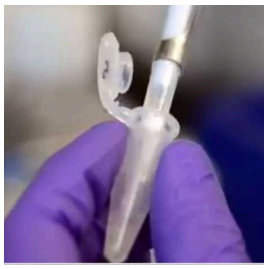
Compartmental Protein Extraction to Achieve Enrichment of Extracellular Matrix (ECM) Proteins



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Human BioMolecular Atl... | The Matrisome Project

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We use this protocol and it's working!

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Abstract

This protocol provides instructions on how to extract cellular components of tissues and enrich for ECM proteins to be later analyzed via immunoblotting or mass spectrometry.

https://www.youtube.com/embed/JrPH9FDY05c

Materials

I. Materials & Reagents

1. Tissue homogenizer: Bullet blender beads **Next Advance Catalog #**BB24-AU OR
Tissue homogenizer: Bead Rupter Elite Beads **Omni International Catalog #**19-042E

2. Subcellular Protein Fractionation Kit for Tissues **Thermo Scientific Catalog #**87790

3. BupH Phosphate Buffered Saline Packs **Thermo Scientific Catalog #**28372

Note

- The protocol uses a series of incubation in buffers of different pH and containing different amounts of salts and detergents to sequentially extract intracellular proteins and enrich for insoluble ECM proteins.
- The volumes of reagents are from Pierce Compartmental given below are for 100mg of tissues or tumors (see Table 1) and need to be adjusted appropriately.
- A cocktail of protease inhibitors (PI, Cat#1862209) is provided as a 100X solution and needs to be added to each buffer. Protease inhibitor: A cocktail of various protease inhibitors is included with the kit, but if this is not used it is advisable to include a variety of inhibitors against cysteine, serine and threonine peptidases, serine esterases, divalent cation-dependent metalloproteinases etc
- We do not recommend conducting the procedure on fixed tissues as fixation (*i.e.*, chemical crosslinking) interferes with decellularization and can also significantly compromise subsequent mass spectrometry analysis.
- For the entire procedure, we recommend the use of low-retention tubes and pipette tips to maximize protein recovery.

Table 1: Volume of reagents from Compartmental Extraction kit to decellularize 100mg (wet wight) of tissue or tumor.

Reagents from Compartment Protein Extraction Kit	Volume for 100mg of tissue	Catalog #'s based on Pierce Subcellular Protein Fractionation kit (Cat#87790)
Buffer CEB[1] + 1X PI[2]	1000µL	Cat#1862568
Buffer MEB[1] + 1X PI[2]	650µL	Cat#1862569
Buffer NEB[1] + 1X PI[2]	225µL x2	Cat#1862570
Buffer NEB[1] + $CaCl_2$ and Micrococcal Nuclease + 1X PI[2]	170µL	Cat#1862570 $CaCl_2$ 100 mM Cat#1862572 Micrococcal Nuclease Cat#1862573
Buffer PEB[1] + 1X PI[2]	125µL	Cat#1862571
1X PBS[1] + 1X PI[2]	600µL/wash	Cat#28372

Troubleshooting

Before start

Note

- The procedure can be conducted on fresh or flash-frozen tissues. We recommend perfusing highly vascularized tissues with PBS at the time of tissue collection to eliminate red blood cells and plasma proteins.

Before starting, prepare the reagents and add protease inhibitors provided with the Subcellular Protein Fractionation Kit for Tissues to the desired volume of each buffer.

All buffers and samples should be kept on ice for the duration of the experiment except the Buffer PEB that needs to be kept at room temperature to prevent SDS precipitation.

Tissue Homogenization

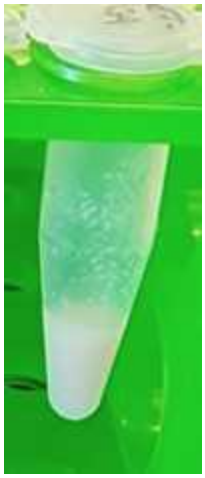
10m

1 Homogenize 100 mg of tissue in 1 mL of ***Buffer CEB*** containing protease inhibitors using a tissue homogenizer until the tissue is completely disrupted and a homogenous suspension is obtained.

Note

- Save a small 50 uL aliquot of the homogenate and add 50 uL of 3X Laemmli buffer with 100 mM DTT to monitor the quality of the protein extraction by western blotting. Flash freeze and store at -80C.

Expected result



Post Homogenization Example

Sequential extraction of intracellular soluble proteins

2h 10m

2 2h 10m

2.1 Extraction of cytosolic proteins: 10m

1. Centrifuge the homogenate at 5000 x g for 00:10:00 at 4 °C .
2. Collect the supernatant in a clean tube: this fraction is enriched for cytosolic (C) proteins. Flash freeze this fraction and store at -80 °C .

Note

- Aliquot 50 uL of the supernatant and add 25 uL of 3X Laemmli Buffer with 100 mM DTT to monitor the quality of the extraction by western blotting. Flash freeze and store at -80 C.

Expected result

Post extraction of cytosolic proteins pellet

2.2 Extraction of membrane proteins: 20m

1. Resuspend the pellet in 650 µL of ***Buffer MEB*** containing protease inhibitors.
2. Incubate the sample on a tube rotator for 00:10:00 at 4 °C
3. Centrifuge the sample at 5000 x g for 00:10:00 at 4 °C
4. Collect the supernatant in a clean tube: this fraction is enriched for membrane (M) proteins. Flash-freeze this fraction and store at -80 °C

Note

- Aliquot 50 uL of the supernatant and add 25 uL of 3X Laemmli Buffer with 100 mM DTT to monitor the quality of the extraction by western blotting. Flash freeze and store at -80 C.

Expected result

Post extraction of membrane proteins pellet

2.3 Extraction of nuclear soluble proteins: 40m

1. Resuspend the pellet in 225 µL of ***Buffer NEB*** containing protease inhibitors.
2. Incubate the sample on a tube rotator for 00:30:00 at 4 °C

Note

- *Buffer NEB* containing protease inhibitors, $CaCl_2$, and micrococcal nuclease should be taken off the ice at this point in preparation for step 2.4.

1. Centrifuge the sample at 5.000 x g for 00:10:00 at 4 °C
2. Collect the supernatant in a clean tube: this fraction is enriched for nuclear soluble (Nsol) proteins. Flash-freeze this fraction and store at -80 °C

Note

- Aliquot 50 uL of the supernatant and add 25 uL of 3X Laemmli Buffer with 100 mM DTT to monitor the quality of the extraction by western blotting. Flash freeze and store at -80 C.

Expected result

Post extraction of nuclear soluble proteins pellet

2.4 Extraction of nuclear chromatin-bound proteins: 40m

1. Resuspend the pellet in 170 µL of ***Buffer NEB*** containing protease inhibitors, $CaCl_2$, and micrococcal nuclease.
2. Incubate the sample on a tube rotator for 00:30:00 at Room temperature

3. Centrifuge the sample at 16000 x g for 00:10:00 at 4 °C
4. Collect the supernatant in a clean tube: this fraction is enriched for nuclear chromatin-bound (Ncb) proteins. Flash-freeze this fraction and store at -80 °C

Note

- Aliquot 50 uL of the supernatant and add 25 uL of 3X Laemmli Buffer with 100 mM DTT to monitor the quality of the extraction by western blotting. Flash freeze and store at -80 C.

Expected result

Post extraction of nuclear insoluble proteins pellet

2.5 30m

Extraction of cytoskeletal proteins:

1. Resuspend the pellet in 125 µL of ***Buffer PEB*** containing protease inhibitors.

2. Incubate the sample on a tube rotator for 00:20:00 at Room temperature .

Note

- Note that the pellet may not fully resuspend. We suggest disrupting the pellet by pipetting up and down until observing disruption of the pellet. To facilitate disruption, we suggest using cut tips.

3. Centrifuge the sample at 16000 x g for 00:10:00 at Room temperature .

4. Collect the supernatant in a clean tube: this fraction is enriched for cytoskeletal (Cs) proteins

Note

- Note at this point a further marked decrease in the size of the pellet (see video available at: **Enrichment of ECM Proteins from Tissues and Digestion into Peptides for Mass Spectrometry Analysis**)
- Aliquot 50 uL of the supernatant and add 25 uL of 3X Laemmli Buffer with 100 mM DTT to monitor the quality of the extraction by western blotting. Flash freeze and store at -80 C.

Expected result

Post extraction of cytoskeletal proteins pellet

Washing the remaining pellet enriched for ECM proteins

10m

3

9m

All traces of detergents need to b 4 °C removed by extensive washes prior to digestion of the proteins into peptides for mass spectrometric analysis. Perform washes as follows:

1. Resuspend the pellet in 600 µL of ***PBS*** containing protease inhibitors.
2. Centrifuge the sample at 16000 x g for 00:03:00 at 4 °C
3. Discard the supernatant
4. Repeat this step two times

Note

- On the final wash, aliquot 150-200 uL of the 600 uL resuspended ECM-enriched pellet to monitor the quality of the extraction by western blotting. Centrifuge aliquot at 16,000 x g for 3 minutes, and resuspend in 25-50 uL of 3x Laemmli buffer with 100 mM of DTT. Flash freeze and store at -80 C.
- Note that the size of the pellet will depend on the amount of insoluble (ECM) proteins in the starting material and the efficiency of the decellularization.

Expected result

Post washing pellet

Sample storage

5m

4 The ECM-enriched pellet can be flash-frozen and kept at -80 °C. The ECM-enriched protein pellet can be subsequently digested into peptides for proteomic analysis: 5m

Protocol

NAME

In-solution Digestion of ECM-Enriched Proteins Samples for Mass Spectrometry Analysis

CREATED BY

Alexandra Naba

Preview

Protocol references

Adapted from Naba *et al.*, JoVE, 2015: https://www.jove.com/t/53057/enrichment-of-extracellular-matrix-proteins-from-tissues-and-digestion-into-peptides-for-mass-spectrometry-analysis