Protein Extraction

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Basic Molecular Pathology Course with "Hands On" Interactive Practice
September 19-21, 2013, Istanbul

Plan

- Samples
- Lysis Buffers
- Equipments and reagents
- My recipe and "Take home messages"

Samples

- Cell lines
- Fine needle aspirates
- Fresh tissues
- FFPE tissues

- When tissues are collected, they should be frozen in dry ice and kept at -80 ^{0}C until they are used.

Lysis and Sample Preparation

- First, the protein from these cells must be solubilized with a strong detergent. The best and most common buffer to solubilize protein for western blotting is Laemmli sample buffer (Named for Professor Ulrich Laemmli).

- Cell disruption can be easily accomplished by freezing-thawing and mechanical shearing with a homogenizer.

- Cell lysis is achieved by mechanical shearing, often with a Dounce homogenizer or by passing cells through a syringe tip.

Buffers

- NP-40 buffer
- RIPA buffer
- Laemmli buffer
- Tris-triton buffer

- Extraction methods must be adjusted according to the nature of the proteins to be studied (e.g. membrane vs cytoplasmic proteins) and the assay to be used (e.g., western blotting vs coimmunoprecipitation)

- If protein-protein interactions are examined by coIP, harsh conditions employing ionic detergents and high concentrations of salt should be avoided.

Protein location	Buffer recommended
• Whole Cell	• NP-40 or RIPA
• Cytoplasmic (soluble)	• Tris-HCl
• Cytoplasmic (cytoskeletal bound)	• Tris-Triton
• Membrane bound	• NP-40 or RIPA
• Nuclear	• RIPA
• Mitochondria	• RIPA

Nonidet-P40 (NP40) buffer

- 150 mM sodium chloride
- 1.0% NP-40 (Triton X-100 can be substituted for NP-40)
- 50 mM Tris, pH 8.0

*This is a popular buffer for studying proteins that are cytoplasmic, or membrane-bound, or for whole cell extracts.

- If there is concern that the protein of interest is not being completely extracted from insoluble material or aggregates,
- RIPA buffer may be more suitable, as it contains ionic detergents that may more readily bring the proteins into solution.

RIPA buffer (Radio Immuno Precipitation Assay buffer)

- 150 mM sodium chloride
- 1.0% NP-40 or Triton X-100
- 50 mM Tris, pH 8.0
- 0.5% sodium deoxycholate
- 0.1% SDS (sodium dodecyl sulphate)

*RIPA buffer is also useful for whole cell extracts and membrane-bound proteins, and may be preferable to NP-40

- or Triton X100-only buffers for extracting nuclear proteins. It will disrupt protein-protein interactions and may
- therefore be problematic for immunoprecipitations/pull down assays.

Tris-HCl buffer

- 20 mM Tris-HCl, pH 7.5

Tris-Triton buffer: (Cytoskeletal proteins)

- 10 mM Tris, pH 7.4
- 100 mM NaCl
- 1 mM EDTA
- 1 mM EGTA
- 1% Triton X-100
- 10% glycerol
- 0.1% SDS
- 0.5% deoxycholate

Buffers

- Extraction buffer:
  - 20mM HEPES pH 7.5
  - 100mM NaCl
  - 0.05% Triton X100
  - 1mM DTT
  - 5mM sodium-β-glycerophosphate
  - 0.5mM sodium orthovanadate
  - 1mM EDTA
  - 0.5mM PMSF
  - 10µg/ml aprotinin
  - 5 µg/ml leupeptin
  - 2 µg/ml pepstatin

Laemmli buffer

- 62.5 mM Tris-HCl pH 6.8
- 2% SDS
- 10% Glycerol
- 5% β-Mercaptoethanol
- .02% Bromophenol Blue

* Laemmli sample buffer is especially formulated for protein sample preparation to be used in the Laemmli SDS-PAGE system.

- All these buffers must be kept at 4^0C for several weeks or for up to a year if aliquotted and stored at -20^0C.

Whole cell lysate preparation (fresh tissue)

- SDS lysis buffer

  2% SDS

  25mM Tris-HCl (pH 6.8)

  5mM EDTA

  1mM PMSF (protease inhibitor-add from stock solution immediately before use)

*All procedures must be done on ice.

To prepare sample;

- add 2-3 volume lysis buffer to 1gr sample and mix well.
- cut the sample to small pieces using surgical knife.
- Homogenize the tissue with 10 to 15 strokes using a teflon homogenizer

Teflon homogenizer

- Transfer the mixture to the clean tube

- Homogenize the tissue using ultrasonicator (during process, avoid from excessive heating-on ice)


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- After homogenisation, centrifuge the cell suspension.

- Cell debris and chromosomal DNA are removed by centrifugation.

- Spin at 15000rpm for 1 hour at 4 °C.
- Transfer the supernatant to a fresh tube (try not to take any lipid from the surface layer or any precipitated particle from the bottom.

- Relative or absolute amounts of total protein must be determined by a total protein quantitation method such as the Bradford method.

- For 1ml of homogenate:
- Add;

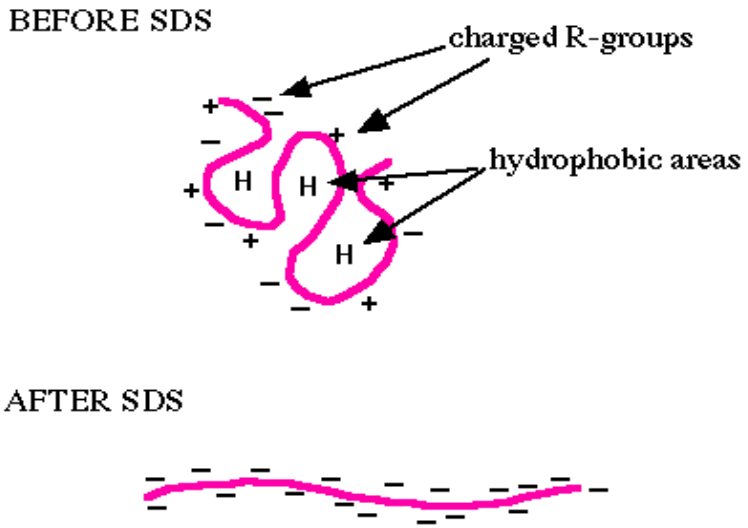
  2µl bromphenol blue

  50 µl DTT

  50 µl Glycerol
- Boil the samples for 5 minutes in a dry block heater.

- Use the samples for electrophoresis or keep at -80 ^{0}C until they are used.

- SDS (sodium dodecyl sulfate) is an ionic detergent applied to protein sample to linearize proteins and to impart a negative charge to linearized proteins.



- PMSF (phenylmethylsulfonyl fluoride) is a serine protease inhibitor.
- It must be dissolved in a small amount of solvent, such as *ethanol, methanol or isopropyl alcohol.
- Effective at 0.1 to 1mM final concentration

*The half-life is short in aqueous solutions. A stock solution of 100mM in ethanol should be made and diluted into buffer immediately before use.

Protease inhibitor	Target Protease
PMSF	Serine proteases
Pepstatin A	Asid proteases
Leupeptin	Thiol proteases
Aprotinin	Serine proteases
EDTA	Metalloproteases

- Metal chelators such as EDTA at a final concentration of 1-5 mM avoid metal-induced oxidation and helps to maintain the protein in a reduced state.

- Glycerol increases the density of the sample so that it will layer in the sample well.

- Bromphenol blue is a low molecular weight dye. It helps to track the progression of gel electrophoresis and sample loading process in the well.

- Reducing agents such as dithiothreitol (DTT) and 2-mercaptoethanol (2-ME) at final concentrations of 1-5 mM also help to maintain the protein in the reduced state by preventing oxidation of cysteines.

"Take Home Messages"

- Temperature and pH must be under control to protect the protein from denaturation.
- All procedures must be done on ice.
- Protease inhibitors must be added.

- Once the concentration of total protein is determined, the extracts must be processed immediately, or frozen and kept at -80 ^{0} C to prevent possible degradation of the protein or deterioration of posttranslational modifications (e.g. phosphate groups)

Equipments and reagents

- Protein source (cells/ tissues)
- Plastic pestles/teflon homogenizer/sonicator
- Lysis buffer/BPB/DTT/Gylcerol
- Eppendorf tubes
- Tube rack
- Dry block heater
- Table-top centrifuge
- Total protein quantitation reagent

Good luck with your protein experiments!

Thank you ..