Product Information

Kit components

A Buffer (RIPA) : 100 ml
B Buffer : 10 ml

[Manufacturer : BDL]

Product Name	Code	Size	Storage
ULTRARIPA kit for Lipid Raft	F015	1 kit	4℃

Outline of procedure

Extract Lipid Raft proteins in 30 minutes!

1. Solubilize samples (tissues / cells) by A Buffer (RIPA)
2. Centrifuge (>10,000 x g, 5 minutes) and collect A buffer insoluble fraction
3. Add B buffer to A buffer insoluble fraction
4. Centrifuge (>10,000 x g, 5 minutes) and collect soluble fraction
5. Use it for further assays!

Advantage of ULTRARIPA kit

	SDS Buffer	RIPA Buffer	ULTRARIPA Kit
Cytoplasm proteins	Can extract but proteins are denatured	Can extract non-denatured proteins	Can extract non-denatured proteins
Membrane proteins (non-Lipid Raft)	Can extract but proteins are denatured	Can extract non-denatured proteins	Can extract non-denatured proteins
Membrane proteins (Lipid Raft)	Can extract but proteins are denatured	Cannot extract	Can extract non-denatured proteins
Immunoprecipitation of Lipid Raft proteins	Not suitable	Not suitable	Suitable
Enzyme activity assay of Lipid Raft proteins	Not suitable	Not suitable	Suitable

FAQ

Q Can ULTRARIPA Kit extract “only” Lipid Raft proteins?

A This product focuses on RIPA-insoluble fraction which contains a lot of Lipid Raft proteins. The main purpose is solubilizing Lipid Raft proteins in RIPA-insoluble fraction.
Please note, it is possible that this kit can extract not only Lipid Raft proteins, but also the other RIPA-insoluble proteins, such as nuclear proteins etc.

Q Does B buffer have higher solubilization efficiency than A buffer?

A Yes, B buffer has higher solubilization activity than A buffer (RIPA).
ULTRARIPA kit recommends “two steps extraction procedure” for the purpose of enrichment and simple purification. However, single extraction step by B buffer also shows higher solubilization efficiency. Please see page 2 “Extraction efficiency by direct addition of B-buffer to sample”.

NOTE
※ All products here are research use only, not for diagnostic use.
※ Specs might be changed for improvement without notice.
※ Numbers after “#” represents product code.
※ Company name and product name are trademark or registered mark.
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BioDynamics Laboratory Inc.

Next Generation RIPA buffer

ULTRARIPA Kit for Lipid Raft

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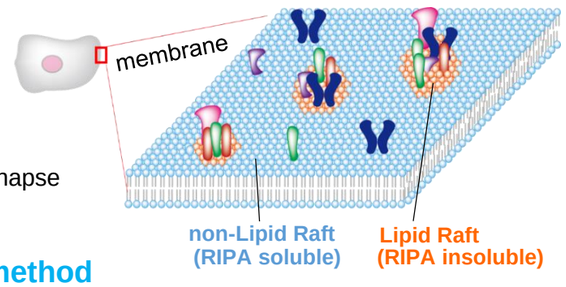
MEMO What is “ Lipid Raft ”?

Lipid raft is a highly specialized microdomain on the lipid bilayer which contains special lipids, cholesterol and functional proteins.

Example of Lipid Raft :
Caveolae, Synapse (Neuron), Immunological synapse

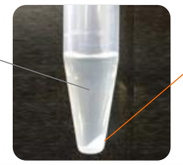


Problems with conventional extraction method

Lipid Rafts are also called as “Detergent Resistant Membrane (DRM)”. Lipid Rafts are usually insoluble by mild detergent buffers such as 1% Triton X-100 and RIPA buffer. SDS can solubilize Lipid Rafts, but the extracted proteins are not suitable for functional assay due to SDS’s strong denaturation ability.
Consequently, it was difficult to analyze functions of lipid raft-associated proteins extracted by these buffers.

Lysed samples by RIPA buffer...

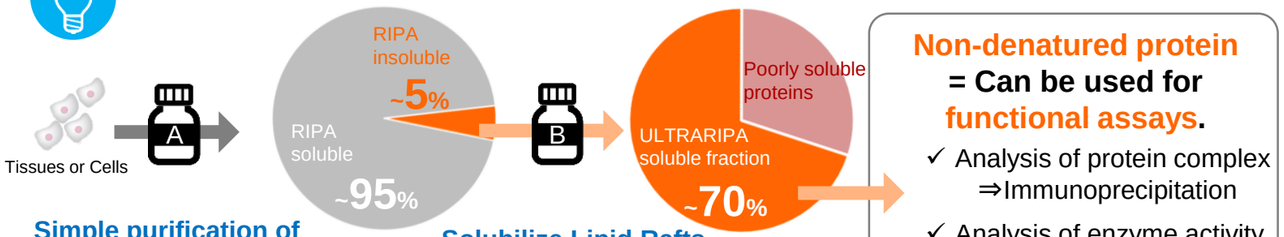
~95%
RIPA-soluble
Contains...
Cytoplasm proteins
Membrane proteins (non-Lipid Raft)



~5%
RIPA-insoluble
Mainly contains...
Membrane Lipid Raft proteins

Proteins in RIPA-insoluble ~5% fraction
✓cannot be solubilized by RIPA
✓SDS can solubilize fraction but solubilized proteins are denatured.
Functional analysis was not possible.

ULTRARIPA kit can help you!



Simple purification of RIPA-insoluble fraction by A buffer (RIPA)

Solubilize Lipid Rafts by B buffer (ULTRARIPA)

Non-denatured protein = Can be used for functional assays.
✓ Analysis of protein complex ⇒Immunoprecipitation
✓ Analysis of enzyme activity ⇒Enzyme assays

✓ Two extraction buffers solubilize Lipid Rafts and concentrate
✓ Less protein denature = Mild extraction
✓ Easy handling : just adding buffers and centrifugation

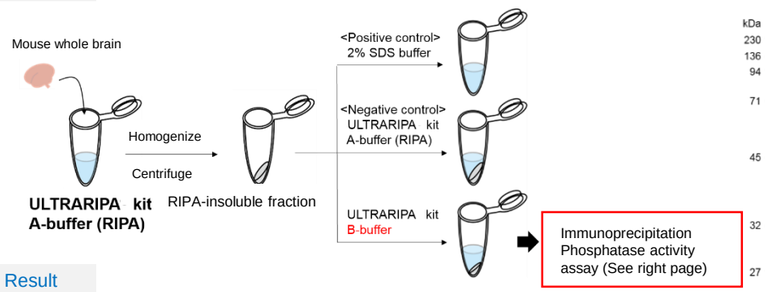
Application Data : See page 2 - 3
Product Detail : See page 4

ULTRARIPA Kit can solubilize Lipid Raft proteins → → → → → → → → Functional analysis of solubilized Lipid Raft proteins

Extraction and functional analysis of the Lipid Raft proteins from mouse brain

Sample Mouse whole brain

Procedure Solubilize RIPA-insoluble fraction by each buffer



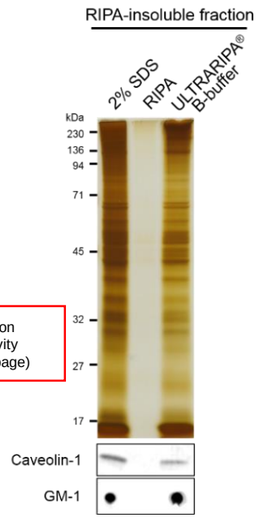
Result

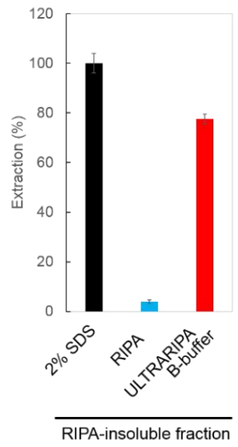
Extracting proteins from RIPA-insoluble fraction

>70% extracted proteins from RIPA-insoluble fraction was observed compared to 2% SDS extraction buffer.

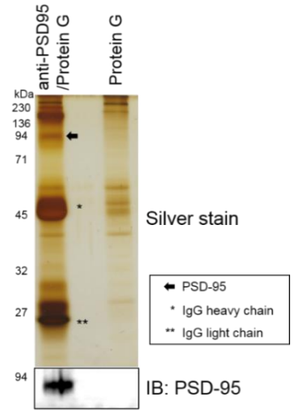
Extraction of Lipid Raft marker (Caveolin-1 and GM-1)

Caveolin-1 and GM-1 were efficiently extracted.





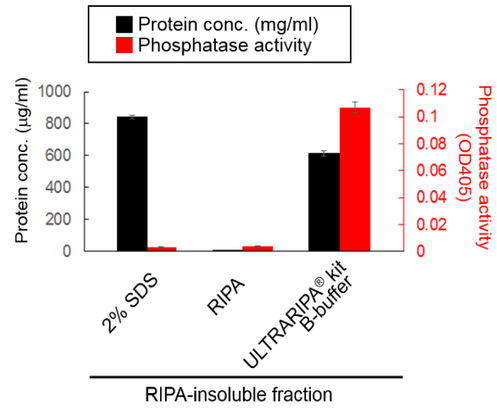
Immunoprecipitation



Antigen-Antibody reaction or Antibody-Protein A/G reaction are not affected

⇒ Compatible with immunoprecipitation

Phosphatase activity assay



Enzyme activity is maintained

⇒ Compatible with enzyme activity assay for RIPA insoluble fraction (≒Lipid Raft protein)

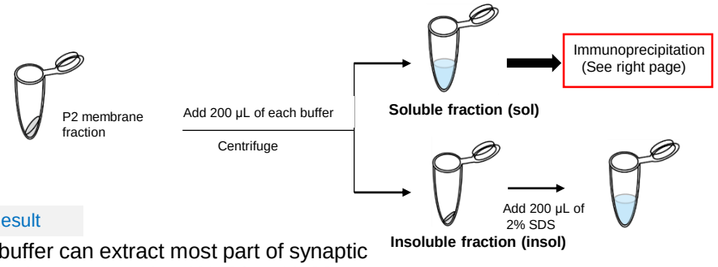
Extraction efficiency by direct addition of B-buffer to sample

Sample P2 membrane fraction of mouse brain tissue (hippocampus + cerebral cortex)

Data : Acquired under the support of Professor Akihiko Takashima and Research associate Dr. Akio Sumioka at Department of Life Science, Gakushuin University.

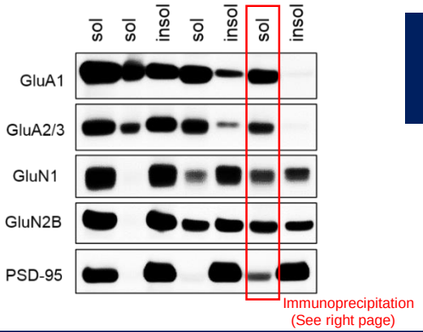
Procedure

1. Solubilize P2 membrane fraction by each buffer
2. Solubilize each insoluble fraction by adding 2%SDS



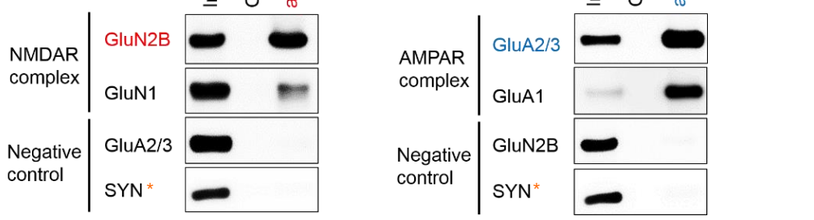
Result

B-buffer can extract most part of synaptic proteins compared to 1% Triton X100 or RIPA buffer (A-buffer).



Analysis of synaptic-protein complex

Immunoprecipitation by using B-buffer soluble fraction



*SYN: Synaptophysin

✓ Physiological NMDAR or AMPAR complex is specifically detected by Immunoprecipitation

✓ Best for analysis of protein complex in RIPA insoluble fraction (≒Lipid Raft protein)

NGF stimulation dependent migration of Integrin to lipid raft

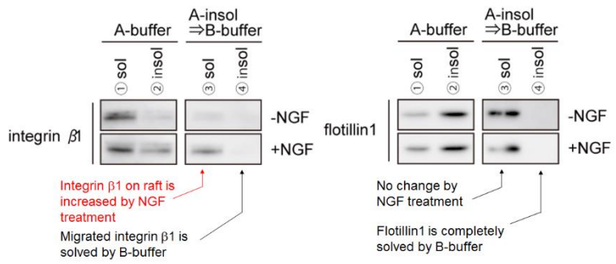
Sample Mouse primary cultured DRG neurons

Data provided by Department of PNS Research National Institute of Neuroscience, NCNP

Procedure Following to ULTRARIPA kit protocol

Result

Flotillin 1 is not changed by NGF stimulation. However, Integrin β1 is accumulated in RIPA-insoluble fraction by NGF stimulation.



Not only detecting the change of RIPA insoluble fraction dependent on external stimulus, but also...

✓ Detecting the change of protein complex dependent on external stimulus

✓ Detecting the change of enzyme activity in RIPA insoluble fraction

ULTRARIPA Kit is the only solution!

1. >70% of proteins from RIPA-insoluble fraction is solubilized under non-denatured condition (against SDS).
2. Maintain enzyme activity of proteins in RIPA-insoluble fraction (≒Lipid Raft protein)
3. No effect on immunoprecipitation – Can detect physiological protein complex of RIPA-insoluble fraction (≒Lipid Raft protein).
4. Detect the change in RIPA-insoluble fraction by external stimulus.