

 **funakoshi**
FRONTIERS IN LIFE SCIENCE
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*BioDynamics
Laboratory Inc.*

Next Generation RIPA buffer

ULTRARIPA Kit for Lipid Raft

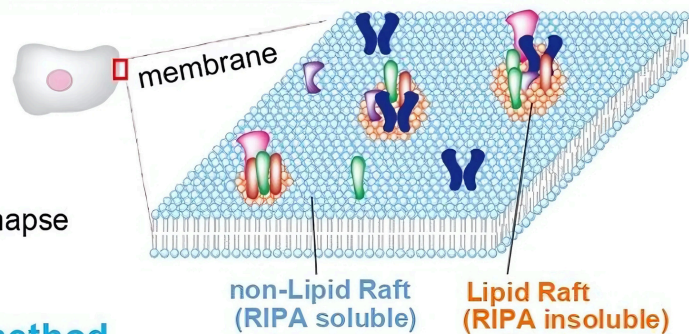


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MEMO What is “Lipid Raft”?

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

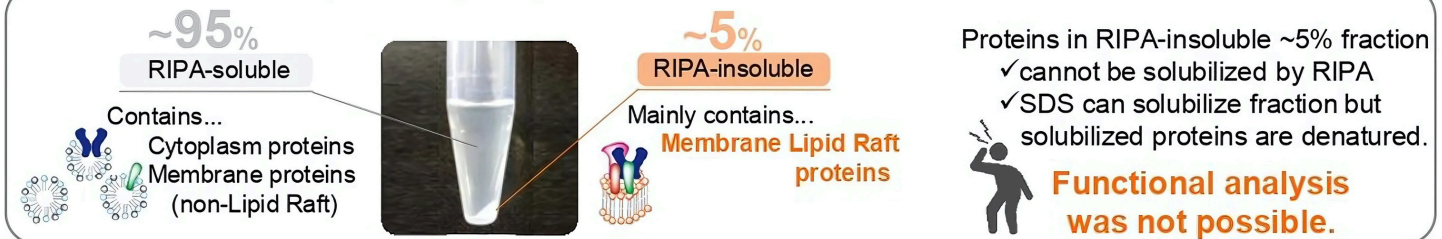


Problems with conventional extraction method

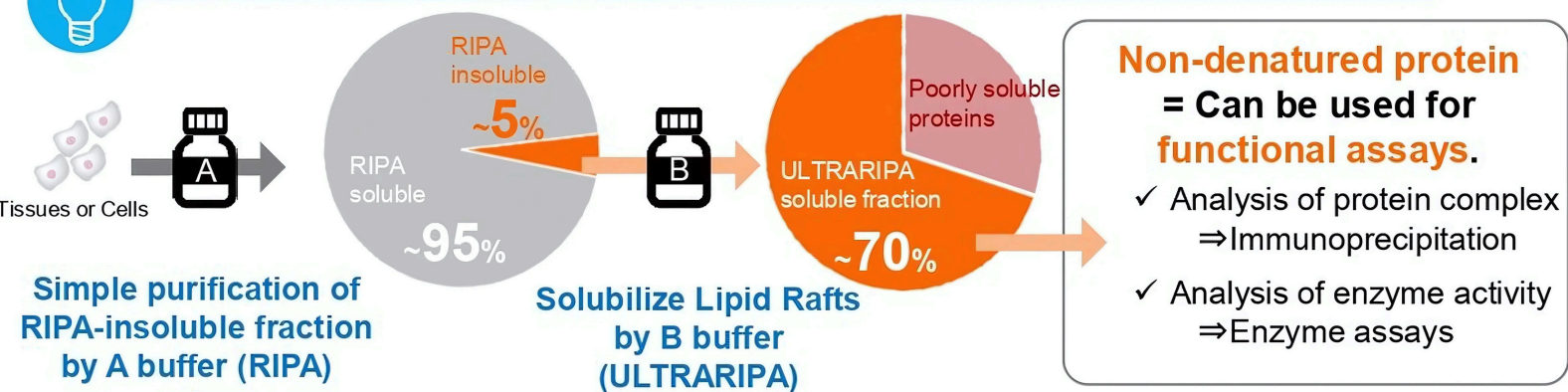
Lipid Rafts are also called as “Detergent Resistant Membrane (DRM)”. Lipid Rafts are usually insoluble by mild detergent buffers such as 1% Triton X-100 and RIPA buffer. SDS can solubilize Lipid Rafts, but the extracted proteins are not suitable for functional assay due to SDS’s strong denaturation ability.

Consequently, it was difficult to analyze functions of lipid raft-associated proteins extracted by these buffers.

Lysed samples by RIPA buffer...



ULTRARIPA kit can help you!



- ✓ 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

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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°C



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

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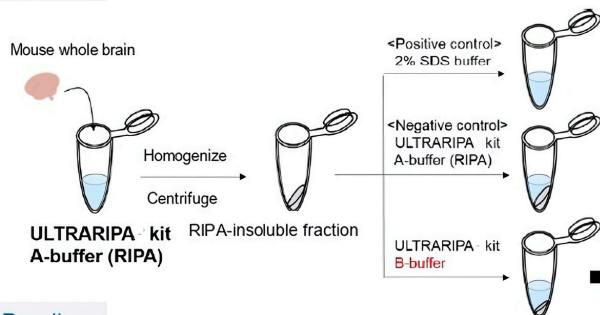
ULTRARIPA Kit can solubilize Lipid Raft proteins



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

Sample Mouse whole brain

Procedure Solubilize RIPA-insoluble fraction by each buffer



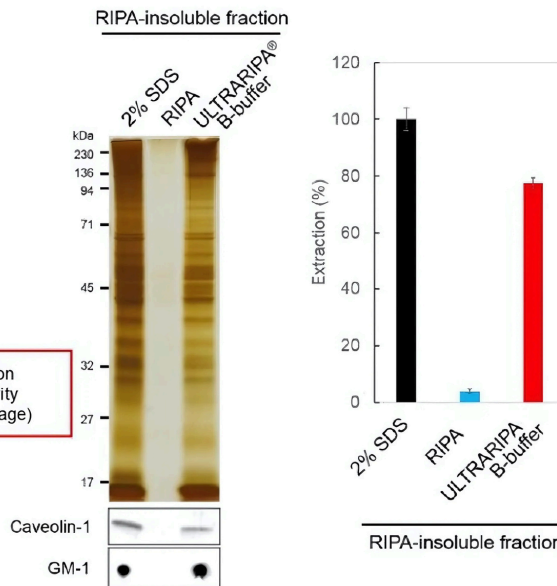
Result

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.

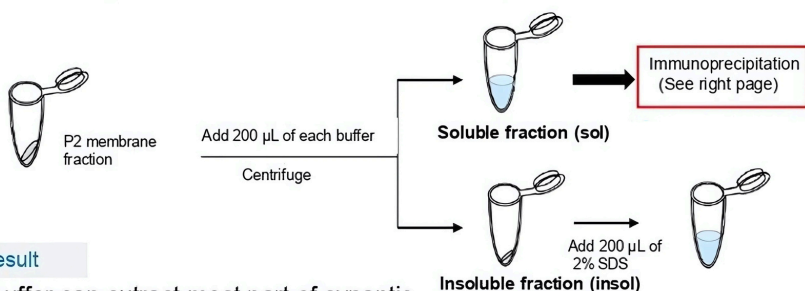


Extraction efficiency by direct addition of B-buffer to sample

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

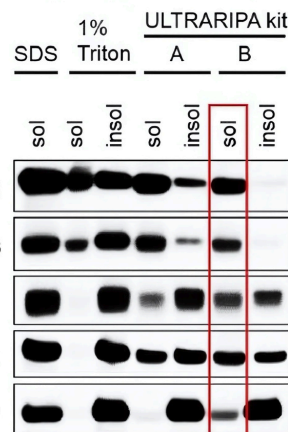
Data : Acquired under the support of Professor Akihiko Takashima and Research associate Dr. Akio Sumioka at Department of Life Science, Gakushuin U

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).



Immunoprecipitation (See right page)

NGF stimulation dependent migration of Integrin to lipid raft

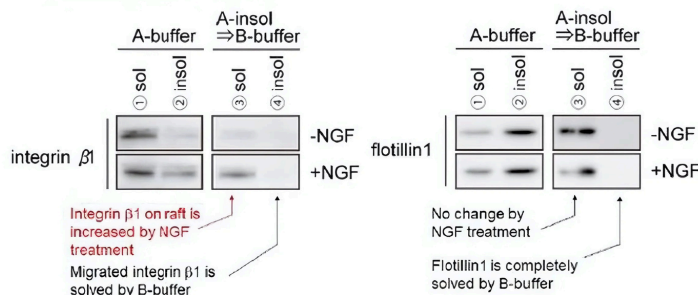
Sample Mouse primary cultured DRG neurons

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 β 1 is accumulated in RIPA-insoluble fraction by NGF stimulation.

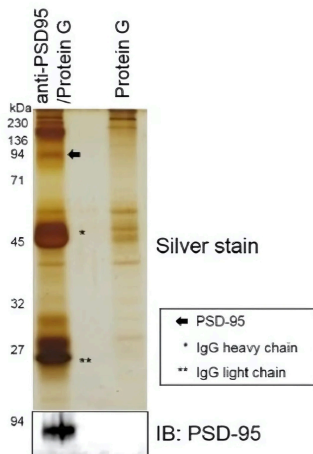


ULTRARIPA Kit is the only solution!

- >70% of proteins from RIPA-insoluble fraction is solubilized under non-denatured conditions
- Maintain enzyme activity of proteins in RIPA-insoluble fraction (\rightleftharpoons Lipid Raft protein)

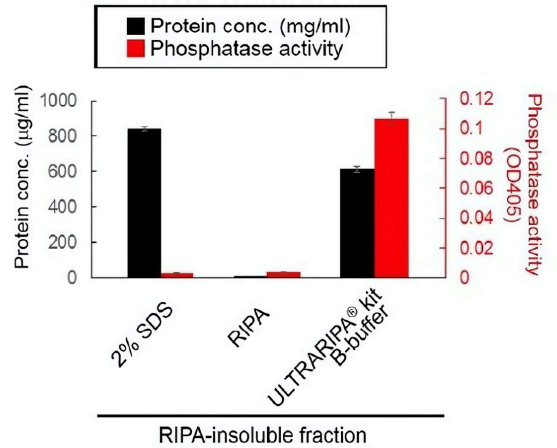
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Immunoprecipitation



Antigen-Antibody reaction or Antibody-Protein A/G reaction are not affected
 ⇒ Compatible with immunoprecipitation

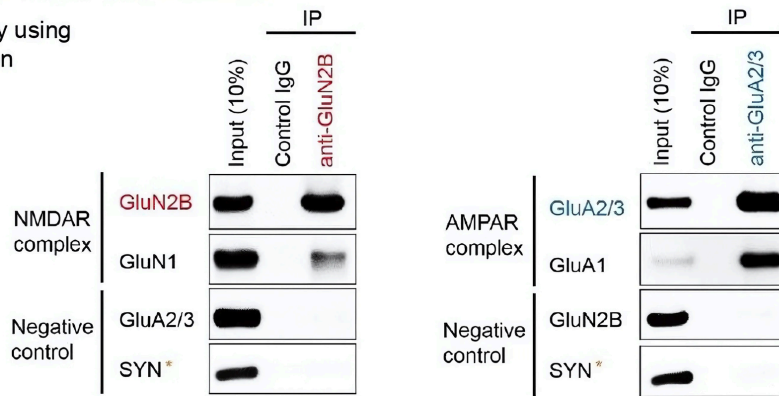
Phosphatase activity assay



Enzyme activity is maintained
 ⇒ Compatible with enzyme activity assay for RIPA insoluble fraction (≡ Lipid Raft protein)

Analysis of synaptic-protein complex

Immunoprecipitation by using B-buffer soluble fraction



Physiological NMDAR or AMPAR complex is specifically detected by Immunoprecipitation
 Best for analysis of protein complex in RIPA insoluble fraction (≡ Lipid Raft protein)

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

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.

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