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
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BDL-1808-F06 (2018.08)

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Next Generation RIPA buffer

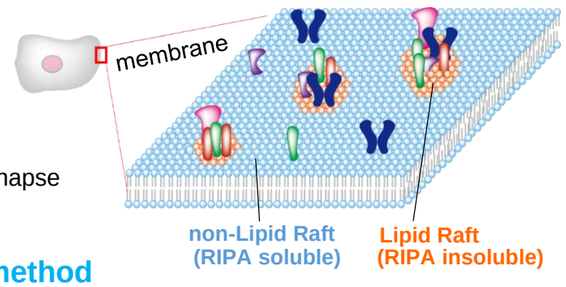
ULTRARIPA Kit for Lipid Raft

What is “ Lipid Raft ”?

MEMO

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

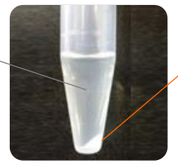


Problems with conventional extraction method

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...

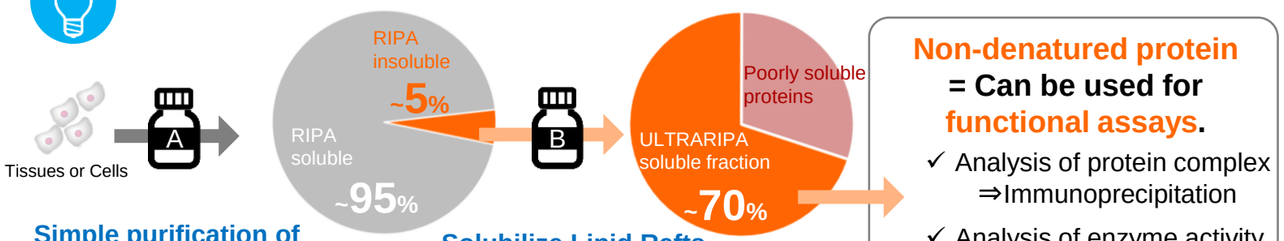
~95%
RIPA-soluble
Contains...
Cytoplasm proteins
Membrane proteins (non-Lipid Raft)



~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

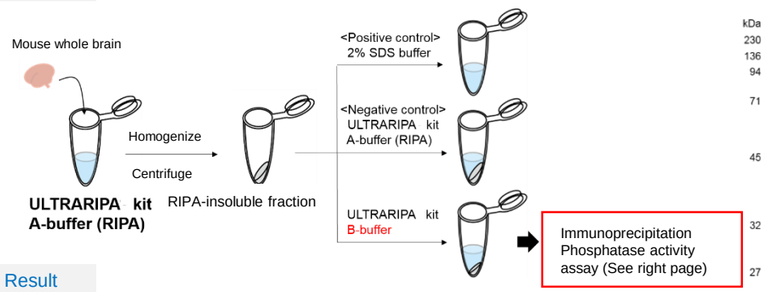
Application Data : See page 2 - 3
Product Detail : See page 4

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



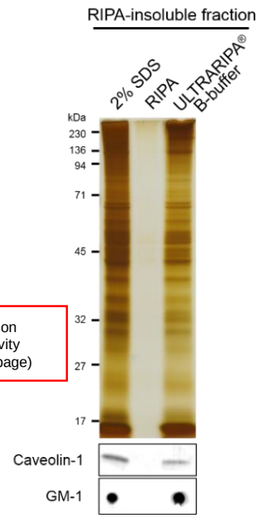
Result

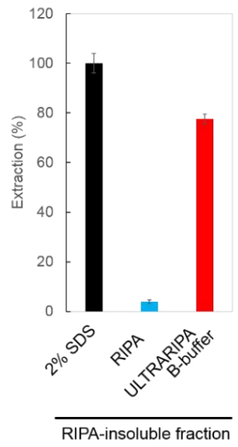
Extracting proteins from RIPA-insoluble fraction

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

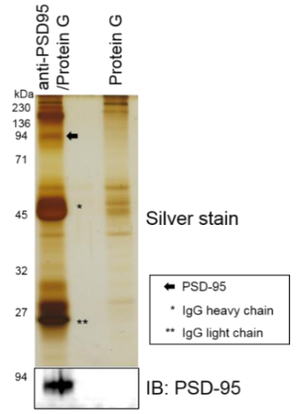
Extraction of Lipid Raft marker (Caveolin-1 and GM-1)

Caveolin-1 and GM-1 were efficiently extracted.





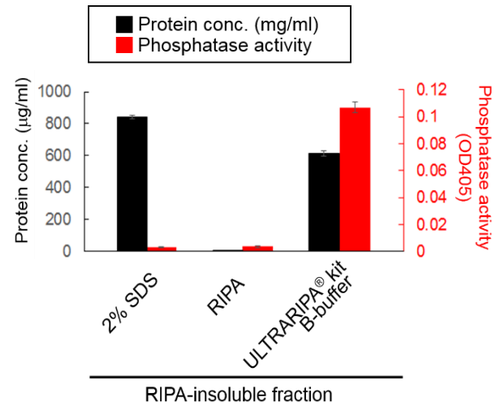
Immunoprecipitation



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)

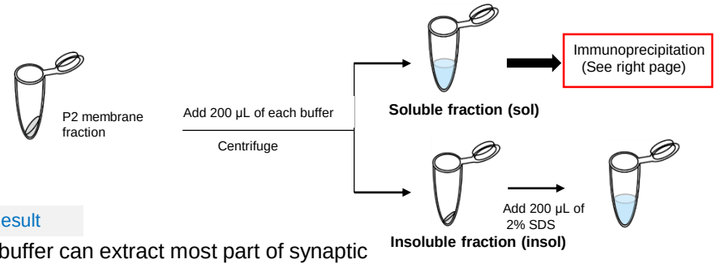
Extraction efficiency by direct addition of B-buffer to sample

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.

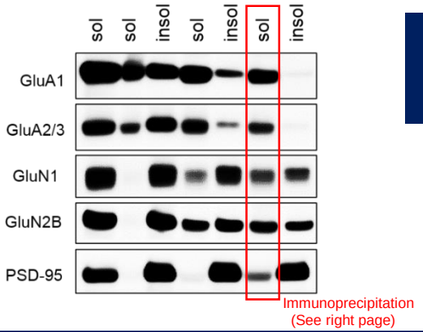
Procedure

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



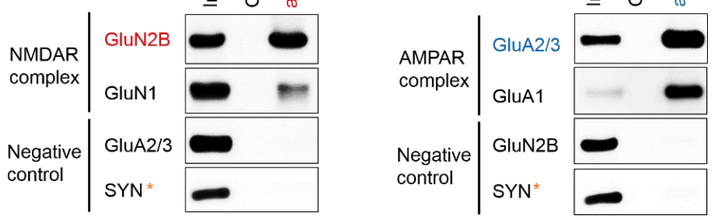
Result

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

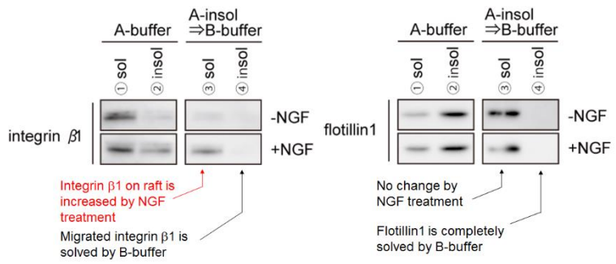
Sample Mouse primary cultured DRG neurons

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

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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.

CliniSciences Group

Netherlands
Company: CliniSciences B.V.
Address: Kraijenhoffstraat 137A,
1018RG Amsterdam, Netherlands
Telephone: +31 85 2082 351
Fax: +31 85 2082 353
Email: nederland@clinisciences.com
Web: http://www.clinisciences.com

Portugal
Company: Quimigen Unipessoal LDA
Address: Rua Almada Negreiros, Lote 6, Loja 14,
2615-275 Alverca Do Ribatejo - Portugal
Telephone: +351 30 8808 050
Fax: +351 30 8808 052
Email: info@quimigen.com
Web: http://www.quimigen.pt

Switzerland
Company: CliniSciences Limited
Address: Markgasse 18 8302 Kloten -
Switzerland
Telephone: +41 (044) 805 76 81
Fax: +41 (044) 805 76 76
Email: switzerland@clinisciences.com
Web: http://www.clinisciences.com

Norway
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1,
Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 084
Email: norge@clinisciences.com
Web: http://www.clinisciences.com

Spain
Company: CliniSciences Lab Solutions
Address: C/ Hermanos del Moral 13
(Bajo E), 28019, Madrid - Spain
Telephone: +34 91 269 40 05
Fax: +34 91 269 40 74
Email: espana@clinisciences.com
Web: http://www.clinisciences.com

UK
Company: CliniSciences Limited
Address: 11 Progress Business center, Whittle
Parkway, SL1 6DQ Slough- United Kingdom
Telephone: +44 (0)1753 066 611
or +44 (0) 330 024 0982
Fax: +44 (0)1753 208 899
Email: uk@clinisciences.com
Web: http://www.clinisciences.com

Denmark
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1,
Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 084
Email: denmark@clinisciences.com
Web: http://www.clinisciences.com

Poland
Company: CliniSciences sp.Z.o.o.
Address: ul. Rolnicza Witolda Pileckiego 67
lok. 209 - 02-781 Warszawa -Poland
Telephone: +48 22 307 0535
Fax: +48 22 307 0532
Email: polska@clinisciences.com
Web: http://www.clinisciences.com

Sweden
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1,
Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 084
Email: sverige@clinisciences.com
Web: http://www.clinisciences.com

USA
Company: Biotrend Chemicals LLC
Address: c/o Carr Riggs Ingram,
600 Grand Boulevard, Suite 210 Miramar
Beach, FL 32550- USA
Telephone: +1 850 650 7790
Fax: +1 850 650 4383
Email: info@biotrend-usa.com
Web: http://www.biotrend-usa.com

Germany
Company: Biotrend Chemikalien GmbH
Address: Wilhelm-Mauser-Str. 41-43,
50827 Köln - Germany
Telephone: +49 221 9480 320
Fax: +49 221 9480 326
Email: info@biotrend.com
Web: http://www.biotrend.com

Austria
Company: CliniSciences GmbH
Address: Stromstrasse 76, A-1180
Wien - Austria
Telephone: +43 720 115 580
Fax: +43 720 115 577
Email: oesterreich@clinisciences.com
Web: http://www.clinisciences.com

Finland
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1,
Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 084
Email: suomi@clinisciences.com
Web: http://www.clinisciences.com

Iceland
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1,
Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 084
Email: island@clinisciences.com
Web: http://www.clinisciences.com

Italy
Company: CliniSciences S.r.l
Address: Via Maremmana Inferiore 378
Roma 00012 Guidonia Montecelio - Italy
Telephone: +39 06 94 80 58 71
Fax: +39 06 94 80 00 21
Email: italia@clinisciences.com
Web: http://www.clinisciences.com

Belgium
Company: CliniSciences S.R.L
Address: Avenue Stalingrad 62, 1000
Bruxelles - Belgium
Telephone: +32 2 31 50 800
Fax: +32 2 31 50 801
Email: belgium@clinisciences.com
Web: http://www.clinisciences.com

France
Company: CliniSciences S.A.S
Address: 74 Rue des Suisses, 92000
Nanterre- France
Telephone: +33 9 77 40 09 09
Fax: +33 9 77 40 10 11
Email: info@clinisciences.com
Web: http://www.clinisciences.com

Ireland
Company: CliniSciences Limited
Address: Ground Floor, 71 lower Baggot street
Dublin D02 P593 - Ireland
Telephone: +353 1 6971 146
Fax: +353 1 6971 147
Email: ireland@clinisciences.com
Web: http://www.clinisciences.com