Product Name: ULTRARIPA® kit for Lipid Raft

Code No. : F015

<Kit components>

A buffer (RIPA buffer) 100 mL
B buffer 10 mL

<Storage condition>

Store at 4°C
Product shelf life : 1 year

<Introduction>

RIPA (**R**adio-**I**mmuno**P**recipitation **A**ssay) **buffer** is one of the most useful buffer for protein extraction. Whereas sodium dodecyl sulphate (SDS)-containing buffer disrupts protein structures, RIPA buffer keeps almost native structures of proteins and can be applied to various applications. However, RIPA buffer is not sufficient to extract membrane proteins and membrane-associated proteins concentrated in lipid raft (Figure 1). Lipid raft is a highly specialized microdomain on the lipid bilayer which contains specialized lipids (sphingomyeline etc), cholesterol and functional proteins. Synapses in neurons, immunosynapses in immunocytes, tight junctions in epithelial cells and focal adhesions in adherent cells are major examples of the lipid raft. These lipid rafts are also called **"Detergent resistant membrane (DRM)**", because lipid raft-enriched proteins usually insoluble by mild detergent buffers such as 1% TritonX100 and RIPA buffer. Consequently, it is difficult to analyze functions of lipid raft-enriched proteins by RIPA buffer.

ULTRARIPA® kit, which consists of two types of detergent containing buffers, can efficiently and rapidly extract membrane proteins/membrane-associated proteins enriched in lipid rafts with native structure and native function. ULTRARIPA® kit is totally new buffer not containing protein denaturing detergents, but can extract the DRM which was hard to extract with RIPA buffer. ULTRARIPA® kit enables to analyze various biological assays of the protein in lipid raft, because enzymatic assay, protein-protein interaction assay etc. is applicable.

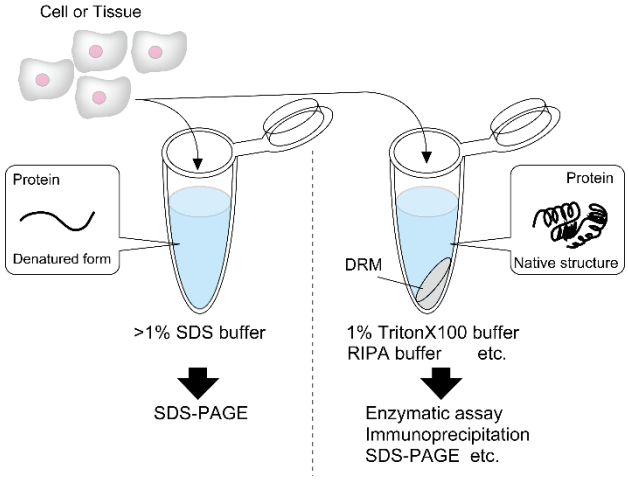

Figure 1: Image of detergent resistant membrane (DRM)

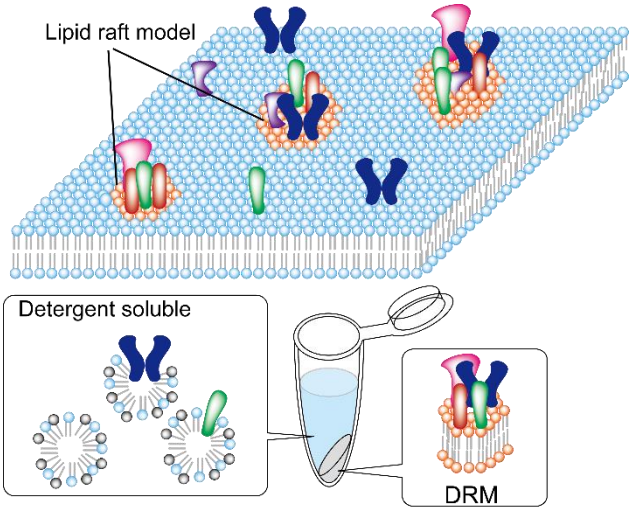

Figure 2: Lipid raft and DRM

<Overview of ULTRARIPA® kit>

ULTRARIPA® kit is 2-step extraction of lipid raft proteins. Firstly, mammalian cells or tissues are dissolved with A-buffer which is the conventional RIPA buffer (50 mM Tris-HCl (pH8.0), 150 mM NaCl, 1% NP-40 alterative, 0.1% SDS and 0.5% sodium deoxycholate). After purification of RIPA-insoluble fraction, lipid raft proteins are extracted by B-buffer. B-buffer enable to effectively solubilize RIPA-insoluble enriched proteins. Both A-buffer- and B-buffer extracts will be able to use for various applications. If you need, the detergent of B-buffer is easily removed by conventional dialysis.

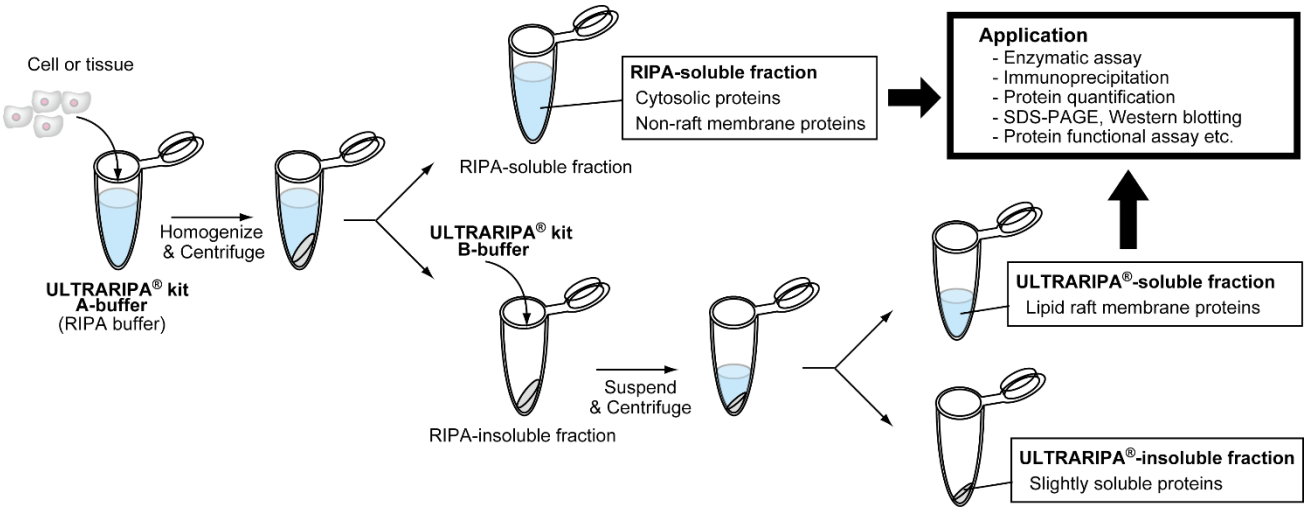

Figure 3: Scheme of UltraRIPA kit

Table Overview of advantages of UltraRIPA kit

	Protein extraction			Protein Structure	Protein Function	Application
	Cytosolic	Membrane				
		Non-lipid raft	Lipid raft			
>1%SDS buffer	○	○	○	×	×	SDS-PAGE
RIPA buffer	○	○	×	○	○	Enzymatic assay Immunoprecipitation SDS-PAGE etc
ULTRARIPA®	○	○	○	○	○	

<Note>

- Neither A nor B-buffer can be applied to Bradford protein assay.
- Please use BCA protein assay if you would like to quantitate protein concentration.

<Experimental procedures>

* Before use :
  - Both A and B-buffer does not contain protease inhibitors. If desired, please add protease inhibitors.
  - B-buffer is stored at 4^{o}C, but please use B-buffer after going back up to room temperature. If ice-cold B-buffer is used for extraction, the extraction efficiency will be dramatically reduced.

○Procedure for mammalian tissue samples

1. Add appropriate volume of ice cold A-buffer to tissue samples (final concentration, 1-10 mg/ml total protein)
2. Homogenize the tissue with a dounce type grinder and a sonicator
   *Sonication is recommended to completely disrupt tissue debris and avoid contamination of nucleus
3. Transfer tissue lysate to 0.5 or 1.5 mL tubes
4. Centrifuge samples at >10,000 xg for 5 min
5. Transfer supernatant to another tube …….**RIPA-soluble fraction**
6. Add 0.5 mL of ice cold A-buffer into the pellet (**RIPA-insoluble fraction**) and vigourously re-suspend the pellet with pipetting or voltax
7. Centrifuge samples at >10,000 xg for 5 min
8. Remove supernatant
9. Add 50-200 μL of B-buffer into the pellet and vigourously re-suspended the pellet with pipetting or voltax at room temperature (option: sonication can be available on ice)
10. Incubate for 5 min at room temperature
11. Centrifuge samples at >10,000 xg for 5 min
12. Collect the supernatant into a new tube…….. **ULTRARIPA®-soluble fraction**

○Procedure for cultured mammalian cells (both adherent cells and floating cells)

1. Remove culture medium from culture dish and wash cells with PBS
2. Add appropriate volume of ice cold A-buffer (for example, 1 mL for 6 well (~10^6 cells), 0.5 mL for 12 well (~5x 10^5 cells)) to the cells with pipetting and sonication and incubate for 10 min on ice
   *Sonication is recommended to completely disrupt cell debris and avoid contamination of nucleus
3. Transfer lysate to 0.5 or 1.5 mL tube
4. Centrifuge samples at >10,000 xg for 5 min
5. Transfer supernatant to another tube…….**RIPA-soluble fraction**
6. Add 0.5 mL of ice cold A-buffer into the pellet (**RIPA-insoluble fraction**) and vigourously re-suspend the pellet with pipetting or voltax
7. Centrifuge samples at >10,000 xg for 5 min
8. Remove supernatant
9. Add 50-200 μL of B-buffer into the pellet and vigourously re-suspended the pellet with pipetting or voltax at room temperature (option: sonication can be available on ice)
10. Incubate for 5 min at room temperature
11. Centrifuge samples at >10,000 xg for 5 min
12. Collect the supernatant into a new tube…….. **ULTRARIPA®-soluble fraction**

<Troubleshooting>

Problem	Possible Cause	Solution
Low RIPA-insoluble fraction yield	Less of total protein	Use more starting cells or tissues
Low concentration of proteins	Excess buffer used	Use less buffer
Degradation of proteins	No protease inhibitors added	Add any protease inhibitors to the both buffers before use

＜**Experimental results**＞

- Extraction of lipid raft proteins from RIPA-insoluble fraction

A whole mouse brain was added into ULTRARIPA® kit A-buffer and fractured by a dounce tissue grinder and a sonicator. After centrifugation of the whole brain lysate, the supernatant (RIPA-soluble fraction) was removed. Then, a pellet (RIPA-insoluble fraction) was washed with A-buffer and re-suspended with ULTRARIPA® B-buffer. 2% SDS and RIPA buffer were used as a positive and a negative control, respectively. Extracted proteins were analyzed by BCA protein assay and western blotting with specific antibodies against lipid raft makers (Figure 4).

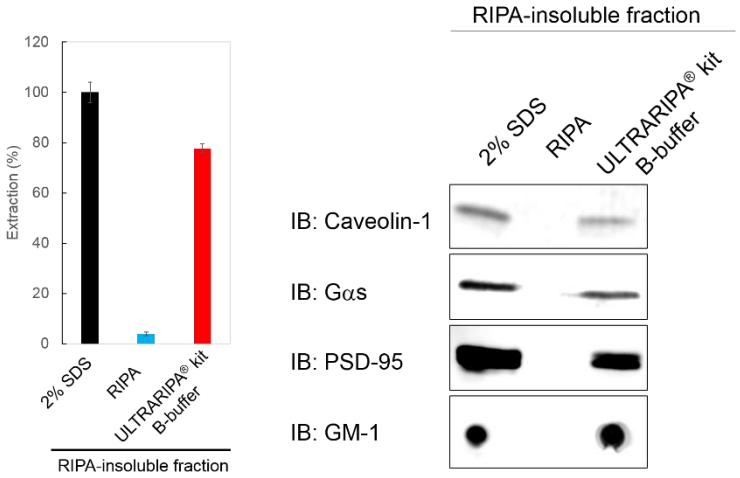

Figure 4: Extraction of RIPA-insoluble proteins by ULTRARIPA® kit

Left: Quantification of extracted total proteins by BCA protein assay. ULTRARIPA® kit could extract constantly over 70% of RIPA-insoluble proteins from the mouse brain tissue.

Right: Western blotting of lipid raft makers. Some lipid raft markers among proteins or a ganglyoside we tested were dramatically extracted from RIPA-insoluble fraction by ULTRARIPA® kit.

- Example of enzymatic assay: Total protein phosphatase activity

Protein extracts from RIPA-insoluble fraction of the mouse whole brain by ULTRARIPA® kit B-buffer, RIPA, and 2% SDS buffer were applied to total protein phosphatase assay (AnaSpec, #AS-71105). Although 2% SDS buffer completely extracted proteins, but it disrupted phosphatases. In contrast, ULTRARIPA® kit showed greatly higher activity of protein phosphatases than 2% SDS and RIPA buffer (Figure 5).

This result means ULTRARIPA® kit is a useful tool to analyze “Uncharacterized enzyme activities” of various lipid raft-enriched proteins.

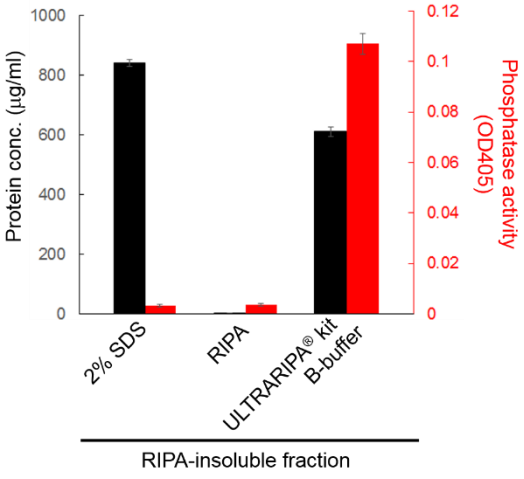

Figure 5: Total protein phosphatase activities in the extract of ULTRARIPA® kit

Black: protein concentration,

Red: phosphatase activity