Invent ® RIPA Lysis Buffer

Cat. No. IN-WB001

Description:

RIPA (Radio-Immunoprecipitation Assay) buffer has been used for protein extraction from mammalian cells and tissues for decades. The buffer contains low concentration of ionic and non-ionic detergents, which usually do not interfere with antigen-antibody binding. The protocol is relatively simple and extracted proteins can be used in applications such as Western blotting, ELISA and immunoprecipitation. Although RIPA buffer has been used widely, it comes with following disadvantages:

1. The protein profile extracted with RIPA buffer is incomplete.
2. Protein loss to the pellet fraction is non-proportional, non-selective and unpredictable.
3. Many artifacts have been reported using RIPA for apoptosis studies.
4. Due to loss of proteins, the amount and ratio of proteins extracted are deviated from those actually present in the cells or tissues resulting in biased data interpretation especially when quantitative and semi-quantitative experiments are involved.

In view of above facts, it is clear that use of RIPA buffer does carry certain risks. If you decide to use RIPA buffer for your research, IN-WB001 is performing as good as any other brands. However, a new technology is available for rapid protein extraction (as fast as 1 min) without artificially altered protein profile. We strongly recommend this state of the art spin column-based technology for routine protein extraction. For more information please visit:
https://www.inventchina.cn/pro_view.asp?id=85&class=1

Contents:

10 mM Tris-HCl (pH 8.0),150 mM NaCl,1% Triton X-100,0.5% Sodium deoxycholate,0.1% SDS, 1mM EDTA (pH 8.0)

Reagents and equipment required：

Centrifuge, microcentrifuge tubes, tissue homogenizer, washing buffers such as PBS and proteinase or phosphatase inhibitors.

Shipping and Storage: Ship at ambient temperature and stored at 4^{o}C.

Protocol：

RIPA Buffer does not contain protease or phosphatase inhibitors. Add proteinase inhibitor to RIPA buffer prior to use. If phosphoproteins are involved add phosphatase inhibitor to the buffer.

For lysis of cultured cells

1. Harvest cultured cells in suspension by low speed centrifugation (500 X g for 5 min). For adherent cells harvest cells by scraping or trypsinization and centrifuge by low speed centrifugation. Wash the cell pellet with cold PBS twice. Remove supernatant completely.

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Note: For adherent cells RIPA buffer can be added directly to culture container after washing with PBS. Gather the lysate to one side using a cell scraper, collect the lysate and transfer to a microcentrifuge tube. Then go to step 3 and 4.

2. Resuspend the cell pellet in cold RIPA buffer by pipetting up and down (1 ml RIPA for 1-5 X 10^7 cells).
3. Incubate the lysate on ice for 5-30 min. Vortex the tube briefly and centrifuge at 4°C,8,000 X g for 10 min.
4. Transfer the supernatant (extracted protein) to a fresh tube and use for downstream experiment or store at -80°C for future use.

For lysis of tissues

Place a piece of fresh or frozen animal tissue in a microcentrifuge tube. Add cold RIPA buffer to cover the tissue (1 ml RIPA for 20-100 mg tissues). Proteinase inhibitor/phosphatase inhibitor should be added to RIPA buffer prior to use as described above. Homogenize the tissue with a proper homogenizer such as plastic pestle for 1.5 ml tube for 30s to 1 min. After homogenization follow step 3 and 4 above.

Note: If a mucoid-like aggregate is seen, remove it with a micropipette before centrifugation. For protein concentration determination use BCA or Lowry assay. For immunoprecipitation we recommend to dilute extracted protein 1:2 to 1:3 with IP washing buffer.

RIPA Buffer- The Potential Troubles

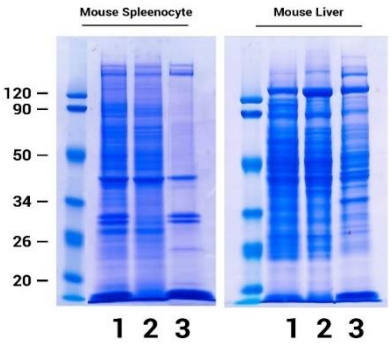
Classical RIPA buffer is comprised of low concentration of sodium dodecyl sulfate (SDS, a denaturing detergent), deoxycholate for disruption of protein-protein interactions and other components. Though different variations of RIPA buffer have been used, protein extraction using RIPA buffer usually generates two distinctive fractions: RIPA-soluble and RIPA-insoluble fractions. Generally, only RIPA-soluble fraction is used for downstream experiments. RIPA-insoluble fraction is discarded. In recent years, more and more researchers have paid a closer attention to the protein components found in RIPA-insoluble fraction and the potential troubles with using RIPA lysis.

Incompleteness of protein profiles

Li [1] compared protein profiles of RIPA-soluble and RIPA-insoluble fraction of mouse splenocytes and liver tissues and a spin-column based commercial kit(Invent Cat#SD-001) and found that protein profiles of RIPA-insoluble fraction are similar but not identical to those found in RIPA-soluble fractions. The proteins lost to the insoluble fraction cover the whole spectrum of protein profiles and the details of protein species found in different RIPA-insoluble fractions vary from sample to sample. The protein loss appears to be unpredictable in different samples. However, the commercial kit is much faster and yields more complete protein profile because there is no insoluble fraction involved.

Based on these results, it is obvious that the protein profile extracted by RIPA lysis is incomplete and altered. Serious data interpretation issues could arise for many qualitative and quantitative experiments using RIPA lysis only.

Comparison of Total Protein Profiles Extracted by Minute™ Total Protein Extraction Kit and RIPA Buffer



Total Proteins were extracted from mouse liver and isolated spleenocytes by MinuteTM Total Protein Extraction Kit (SD-001) and RIPA buffer (R0278, Sigma). Lane 1,2, and 3 are total protein extracted by Minute™ kit, RIPA buffer and proteins from pellet post-RIPA buffer extraction respectively. Proteins in RIPA buffer pellets were extracted by Minute total protein extraction kit (SD-001). Significant amount of proteins was lost in the pellet of RIPA buffer extraction (lane 3).

Uneven loading control

Uneven loading contorl is a common problem in WB experiments. It may be because the expression of House Keeping gene is not constant, or concentration detection is inaccurate, but sample extraction method will also affect it.

To test how RIPA lysis conditions affected immunoblotting results, Kevin et al.[2] lysed HT-29 human colon adenocarcinoma cells in RIPA buffer, boiled the RIPA-insoluble pellet in an equal volume of dithiothreitol-containing Laemmli sample buffer, and then immunoblotted for 20 different protein targets. As expected, RIPA lysis buffer efficiently solubilized many cytoplasmic proteins :GAPDH, Hsp90 and Iκβα, various kinases(Fig.A and B). RIPA lysis also extracted the cytoskeletal and cytoskeleton-associated proteins, actin and focal adhesion kinase (FAK). However, tubulin and intermediate filament proteins (lamin A and KRT5) showed substantial losses into the RIPA-insoluble fraction (Fig.C). Remarkably, RIPA insolubility was not limited to cytoskeletal proteins: The transcription factor GATA2 and the cell-cell adhesion protein β-catenin were also present in the insoluble fraction. In contrast, lysis with Laemmli sample buffer, followed by shearing of the viscous genomic DNA with a high-gauge needle solubilized proteins that are tightly associated with DNA, such as histones (Fig.D). Despite rules of thumb for protein solubility in various lysis buffers, these results show that it is the best to confirm proper solubilization of proteins of interest before embarking on an immunoblot study.

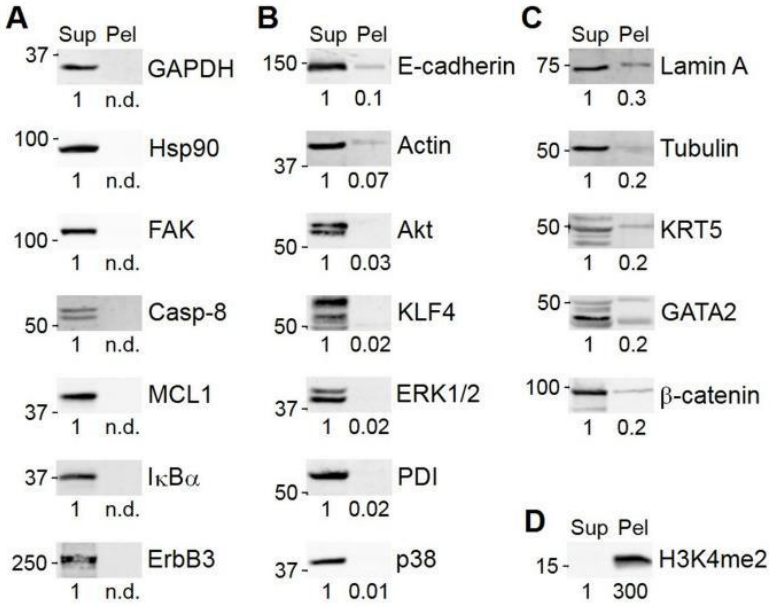
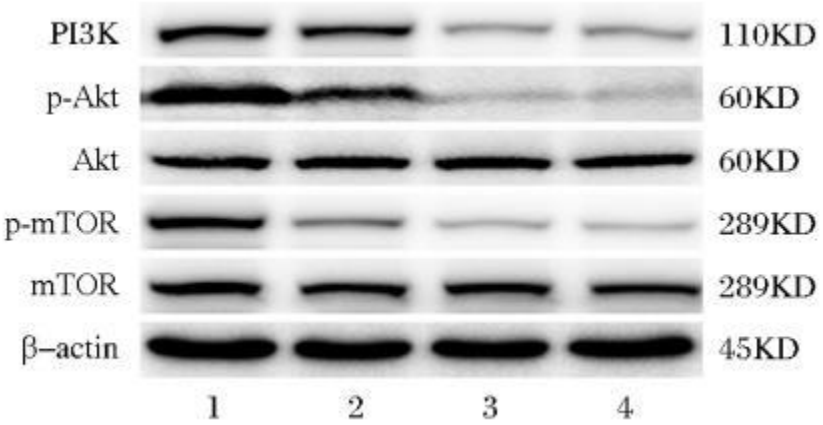


Fig. 1.
Radioimmunoprecipitation assay (RIPA) buffer solubilizes many, but not all, cellular proteins. (**A**) Examples of proteins that are entirely solubilized (100% in the supernatant, Sup). (**B**) Examples of proteins that are mostly solubilized (>90% Sup). (**C**) Examples of proteins that are partially solubilized (≤90% Sup). (**D**) Dimethyl-lysine 4 histone H3 (H3K4me2) resides almost entirely in the RIPA-insoluble pellet (Pel). Band intensities were quantified from the 16-bit digital image by densitometry in ImageJ and are shown normalized to the Sup lane for each target. n.d., not detected. Data are representative of 2–4 experiments.

Affect phosphorylation detection

Ni Hui et al. [3] studied the effects of different protein extraction methods on the expression of PI3K / Akt / mTOR signal pathway related proteins. Bilateral hippocampal tissues were taken out from three rats in the blank group and three rats in the model group. Each sample was extracted by RIPA lysis method and Invent spin-column based protein extraction method. The expression of PI3K / Akt / mTOR signal pathway related proteins was detected by WB.



Electrophoresis of PI3K / Akt / mTOR signal pathway related proteins in two groups of rats with different protein extraction methods

1: Blank +Invent group; 2: Model + Invent group; 3: Blank + RIPA group; 4: Model + RIPA group

The results showed that the expression levels of PI3K/β-actin, p-Akt/Akt and p-mTOR / mTOR in RIPA extraction method were significantly lower than those of Invent group. In the Invent spin-column based extraction method, there was significant difference in the expression level of PI3K/β-actin, p-Akt/Akt and p-mTOR / mTOR between the blank group and the model group ($P = 0.001$), the expression level of PI3K/β-actin, p-Akt/Akt and p-mTOR / mTOR in the model group was significantly lower than that in the blank group; However, there was no significant difference in the expression level of PI3K/β-actin, p-Akt/Akt and p-mTOR / mTOR between the two groups in RIPA extraction method ($P > 0.05$).

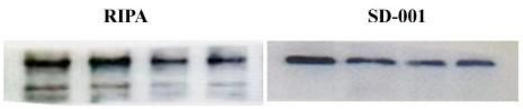
The expression of phosphorylated protein is very low and accounts for a small part of the total protein. It is easy to degraded during protein extraction or rapidly dephosphorylated due to improper processing, which often leads to unsatisfactory results of the phosphorylated protein in Western Blot detection. If part of the protein is lost during protein extraction, the ratio between phosphorylated and non-phosphorylated proteins can be altered, leading to data bias.

An Unexpected Factor That Causes Higher Background in Western Blots

Many factors can contribute to high background in WB, which include but not limited to improperly diluted antibodies, insufficient blocking, interference from blocking reagents, insufficient washing and poor quality of transfer membranes. **In addition to above mentioned factors another factor that affects the background in WB is the method/reagent used for protein extraction/sample preparation. When trouble shoots for high background in WB few researchers realize that the methods for protein extraction can significantly impact the background.** Following two figures show a side by side comparison of the effect of protein extraction methods on WB background. RIPA buffer was compared with a Minute protein extraction kit from Invent Biotechnologies.

Comparison of Signal/Noise Ratio of RIPA buffer and SD-001

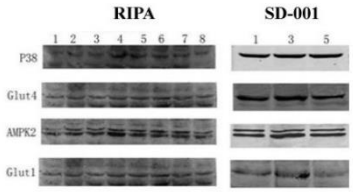
Courtesey of Chengdu University of Chinese Medicine



Total proteins were extracted from Zebrafish with RIPA buffer or Total protein extraction kit (SD-001). Equal amount of proteins were loaded on to 4 lanes and probed with anti-GAPDH with the same dilution. The background is much cleaner with SD-001 extracted sample.

Comparison of Signal/Noise Ratio of RIPA Buffer and SD-001

Courtesy of Bio-platform Shenyang China



Total proteins were extracted from mouse heart tissue with RIPA buffer or Minute total protein extraction kit (SD-001). Equal amount of extracted protein was loaded onto SDS-PAGE and probed with 4 different antibodies in Western blotting using the same protocol and antibody dilution. SD-001 shows stronger signal intensity and much cleaner background.

Conclusion： Protein extraction methods significantly impact the background of WB and Minute kit are superior to RIPA lysis for Western blot.

If you encounter any of the above problems while using RIPA lysis, we strongly recommend this state of the art spin column-based technology for routine protein extraction. For more information please visit: https://www.inventchina.cn/pro_view.asp?id=85&class=1

References:

1. Li, Q. (2016) Pitfalls of Protein Extraction by RIPA Buffer. BioTechniques.Biotechniques. 61:327

2. Kevin A. Janes,2017, An analysis of critical factors for quantitative immunoblotting. Sci Signal.; 8(371): rs2. doi:10.1126/scisignal.2005966.

3. Hui N I, Zhang M, Haiqing A O, et al. Effect of Different Protein Extraction Methods on Expressions of PI3K/Akt/mTOR Signaling Pathway-related Proteins[J]. Chinese Archives of Traditional Chinese Medicine, 2019.

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