RIPA Buffer- The Potential Troubles

(Invent Biotechnologies Inc.)

Radioimmunoprecipitation assay buffer (RIPA buffer) is the most commonly used buffer for total protein extraction from vertebrate cultured cells and tissues [1] Extracted proteins can be used for a variety of downstream applications such as SDS-PAGE, Western blotting, nucleic acid binding, immunoprecipitation, co-precipitation, ELISA, enzymatic activity assay and affinity-based protein purifications. Due to the large heterogeneity of proteins and interfering contaminants, it is a great challenge to simultaneous release and solubilize all proteins in a given sample. Integration of proteins into membranes and the formation of complexes with other proteins or nucleic acids in cells hamper the extraction process significantly. As a consequence, extracted proteins are likely to be more or less distorted compared to in vivo populations. RIPA buffer has been used and regarded as a "gold standard" for total protein extraction for more than two decades due to its cell lysis ability and its suitability for protein quantification and compatibility to proteinase/phosphatase inhibitors. However, there is a lack of systemic evaluation of the efficacy of RIPA buffer in terms of extraction efficiency and completeness of extracted protein profiles. This mini review focuses on potential problems of using RIPA buffer for total protein extraction and its general use in downstream experiments.

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. It is found that certain proteins are more soluble in RIPA buffer than others. Fibronectin from F9 aggregated was found poorly solubilized in RIPA buffer [2] and equal amount of Septin 7 [3]. from mouse front cortex sample was found in both RIPA-soluble and RIPA-insoluble fractions indicating that the efficiency of protein extraction by RIPA buffer is low for these particular proteins. In recent years, more and more researchers have paid a closer attention to the protein components found in RIPA-insoluble fraction and their effects on experimental results and overall data interpretation. Bai and Laiho extracted proteins from Hela cell nucleoli by RIPA buffer and found that the protein profiles of soluble and insoluble fraction are quite different indicating the protein loss is not proportional [4]. Mukhopadhyay et al. [5] extracted total proteins from mutant mouse mammary epithelial cells and found that EGFR, HSP90, c-Src, and tubulin were easily detected by Western blotting in RIPA-insoluble fraction suggesting these proteins are quite abundant in the RIPA-insoluble fraction. Wang et al. [6] compared an SDS-heat method and RIPA buffer for extracting proteins from Zebrafish liver tumors and found that RIPA buffer shows much poorer efficacy for extracting high molecular weight proteins in the samples. Li [7] compared protein profiles of RIPA-soluble and RIPA-insoluble fraction of mouse splenocytes and liver tissues and a spin-column based commercial kit 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.

Ngoka [8] reported a side by side comparison of protein profiles of RIPA-soluble and RIPA-insoluble fractions from several groups of breast cancer samples by mass spectrometry. It was found that the average molecular weight of proteins extracted from RIPA insoluble fractions using a urea-based buffer is about 60% higher than that of RIPA-soluble fractions. In other word, many high molecular weight proteins are lost to the RIPA-insoluble fractions. It was also shown that nearly all extracellular matrix proteins (ECM) and many cytoskeleton proteins were found in RIPA-insoluble fractions. These results indicate that the protein profile extracted by RIPA buffer is incomplete and somewhat biased due to protein loss to the insoluble fraction. One of the major applications using RIPA derived cell lysate is to perform qualitative and/or quantitative analysis of target proteins [9,10,11]. A comprehensive analysis of critical factors affecting quantitative immunoblotting was reported recently [12]. In this study, tubulin, lamin A, KRT5 showed substantial losses into the RIPA-insoluble fraction. Most remarkably, histone protein H3K4me2 was found exclusively in RIPA-insoluble fraction. The transcription factor GATA-2 and adhesion molecule B-catenin were also present in the insoluble fraction. Another important founding from this study is that sample preparation methods significantly impact the experimental results. Janes [12] compared the effects of cell lysates derived from NP-40, RIPA buffer and Laemmli buffer on detection of cleaved form of caspase-8 by Western blotting and found that it was only detected in the cell lysate prepared with Laemmli buffer and it was not detectable in RIPA buffer-derived cell lysate indicating that RIPA buffer is not suitable for this type of analysis.

Cell lysate from RIPA buffer extraction was found to artificially increase the activity of certain protein kinases. The in vitro protein kinase activity derived from RIPA buffer lysate of colon cancer cells was found to be elevated five-seven folds as compared to the activity from the same cells using NP-40 as cell lysis reagent [13]. Zapata et al. [14] compared caspase activation using RIPA buffer extracted cell lysate from activated T-lymphocytes with that of extracted from 2%SDS and found that RIPA buffer artificially activates caspase-3 via releasing GraB from cell lysate. These results strongly suggest that great precaution must be taken for data interpretation when RIPA buffer is used.

As discussed above, total protein extraction using RIPA buffer suffers many disadvantages because of incomplete protein extraction. The loss of protein in the extraction process would result in altered protein profiles in terms of total protein species, ratio between different protein species and activity of certain proteins. The intensity of Western blot using RIPA-extracted lysate could be artificially enhanced or reduced. It has been shown [12] that 10-30% protein was lost in RIPA-insoluble fraction. If the protein lose is consistent and predictable then the choice of sample

preparation methods would not be critical. However, if specific proteins can shift between soluble and insoluble fractions in a cell/tissue type or stimulated state-dependent manner as found in the case of caspase-8 [12] then, the data interpretation could become a major problem. Due to the loss of proteins into the RIPA insoluble fraction, minimize the adverse effects is a challenging task that generally involves careful evaluation and selection of different protein extraction methods. An ideal total protein extraction is to obtain a protein profile that faithfully reflects all protein species present in a given sample with a ratio that is a reflection of true protein ratio present in the sample. Most researchers believe that the method they employed for total protein extraction is a reflection of what present in vivo but the pitfalls are few people actually perform a side by side comparison of different protocols. In many cases, the chances are the protein profile may not be as complete as many researchers like to believe. Therefore, a total protein extraction protocol with maximum protein solubility, minimum protein lose and a complete protein profile should be the best choice.

References

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