© Invent Biotechnologies, Inc.

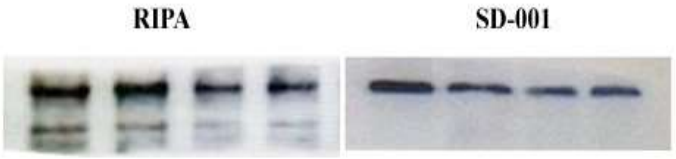
An Unexpected Factor That Causes Higher Background in Western Blots

Invent Biotechnologies Inc.

The Western blot (WB) is a commonly used technique for protein analysis. With this single assay, individual proteins can be assessed for molecular weight, protein modifications, relative abundance and protein localization and interactions. The key for a successful WB is to use an antibody that specifically reacts with the target protein without significant cross-reactivity. A successful blot also relies on a signal/noise ratio that allows detection of the protein with minimal background. High background is a very common problem 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.

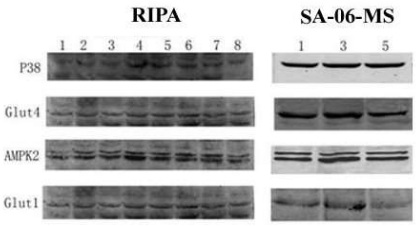
Comparison of Signal/Noise Ratio of RIPA buffer and SD-001



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 SA-06-MS

Courtesy of Bio-platform Shenyang China



Total proteins were extracted from mouse heart tissue with RIPA buffer or Minute total protein extraction kit (SA-06-MS). 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. SA-06-MS shows much cleaner bacground.

Conclusion: Protein extraction methods significantly impact the background of WB and Minute™ kits are superior to RIPA buffer for Western blots.