



# SIMPLE WESTERN ASSAY (automatic western blot)

## ABOUT SIMPLE WESTERN ASSAYS

Simple Western is a capillary based nano-proteomic immunoassay, and an automatic Western Blot with high through-put capability. It is a hands-free, gel-free, mess-free alternative to the labor-intensive traditional western blot.

## CAPABILITIES

- Quantitation of absolute or relative amounts of a protein of interest
- Assay development for novel antigens/targets
- Separation of proteins based on either sizes (MW) or electric charges (pI)
- Quantitation of multiple target proteins / biomarkers in samples of limit amount, such as those from cells of live animals and patients including fine needle aspirates, laser capture microdissection, cell culture, etc
- Real-time toxicological and PK/PD monitoring drug candidates in experimental animals
- Monitoring signaling transduction events in limited samples (as low as 25 cells),

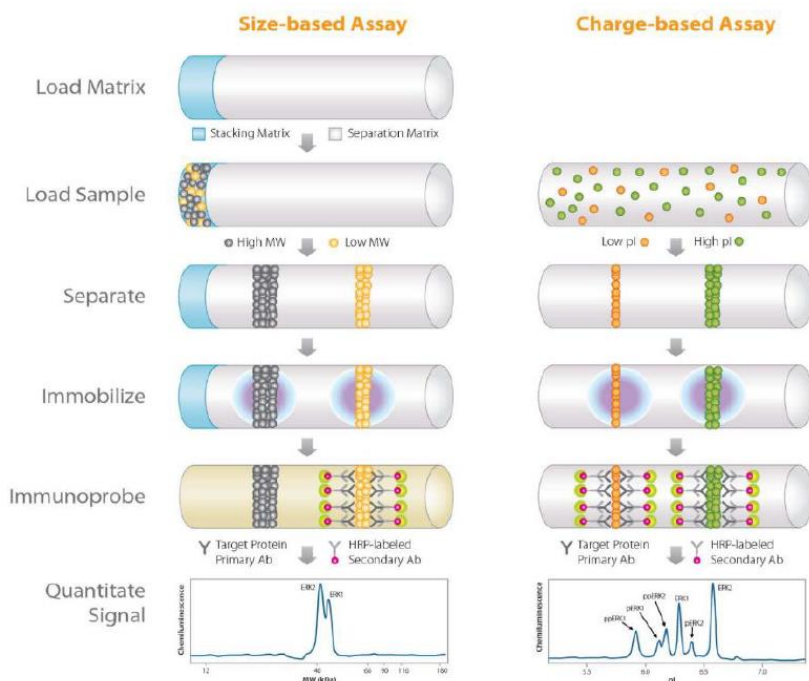
- great for those from stem cells, bone marrow cells, sorted cells, etc
- Discovery and validation of biomarkers to support developing predictive and companion diagnostics
- Characterization of monoclonal and polyclonal antibody binding affinities
- Characterization of relative specificity of antibody to the target protein of interest
- Quantitation of protein phosphorylation even without phospho-specific antibodies
- Phospho-protein profiling and quantitation of post-translational protein modifications using pan antibodies (after protein separation by charge/pI value) or using isoform specific antibodies (after protein separation by size/MW)
- Screening test compounds for effects on expression or modifications of any protein or signaling molecule of interest

## SAMPLE REQUIREMENTS

- Samples should be BSL- I or II
- Samples (about > 10  $\mu$ l of 2 or 4 mg/ml protein) should be submitted
- Any extraction procedures are the responsibility of the researchers. Special arrangements can be made in advance
- Submit samples in a minimum-binding microfuge tubes, accompanied by a template that outlines the sample order
- Ship samples in dry ice



## HOW DOES OUR ASSAY WORK?



**This process is fully automated inside the capillary!**

Unlike a traditional western, there is **NO**:

Gel or apparatus preparation

Transfer to a membrane

Manual incubation or wash steps

Developing/wasting film

Subjective data analysis

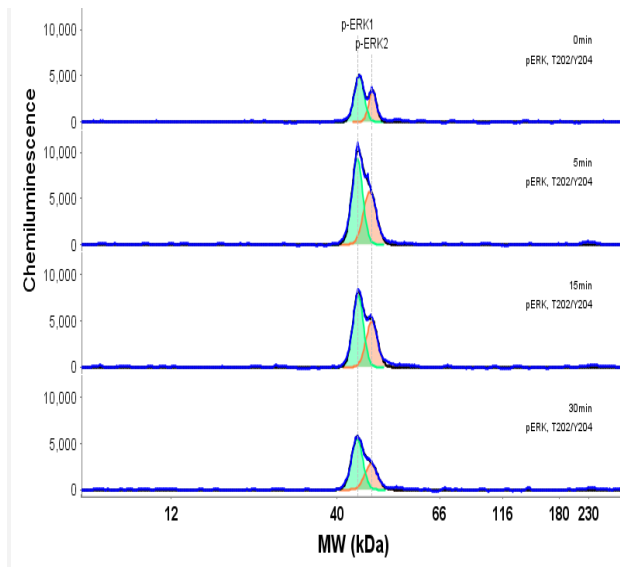
## SERVICE FEATURES

- ❖ **Most sensitive and Automatic** Western blot to examine proteins in a minimal amount sample
- ❖ **Multiplex**
- ❖ **Qualitative and quantitative**
- ❖ **Highly reproducible**
- ❖ **Flexible:** we can quantify any protein of interest, even of rare or low abundance, in samples of limited amount (as low as 25 cells),
- ❖ **Minimal samples:** Samples can be those from cells of live animals and patients including fine needle aspirates (FNA), laser capture microdissection (LCM), FFPE samples, sorted cells, as well as serum, plasma, saliva, urine, etc.
- ❖ **High throughput:** 24 or 96-well format
- ❖ **State-of-art platforms:** Wes, Peggy.

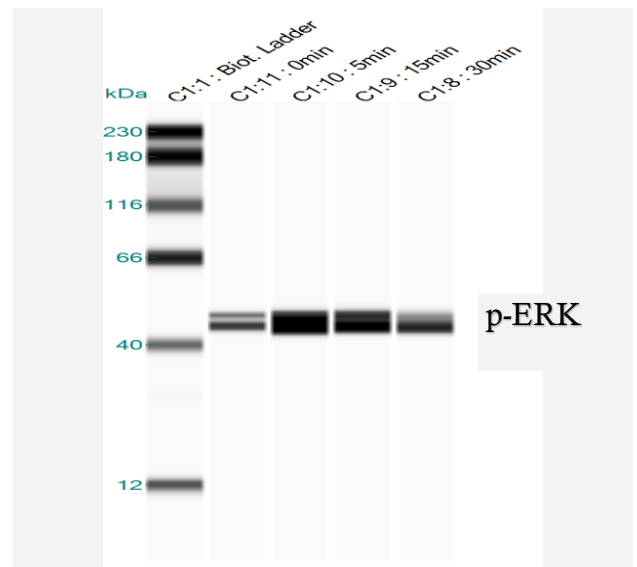
SKU: SIMPLE WESTERN Assay

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- ❖ **Timely data delivery:** 1-4 weeks or sooner, upon receiving test samples/ primary antibodies
- ❖ **20+ years of experience:** Expert data analysis and interpretation, high quality scientific and technical support
- ❖ **Optional tests**
  - ✓ We have a library of >3000 validated antibodies to examine target proteins
  - ✓ We offer ~100 human cancer cell lines and primary cells for testing drugs and biologicals for specific projects
  - ✓ we do homogenization and preparation of tissue lysates
  - ✓ we do protein concentration measurement/ normalization using assays such as BCA

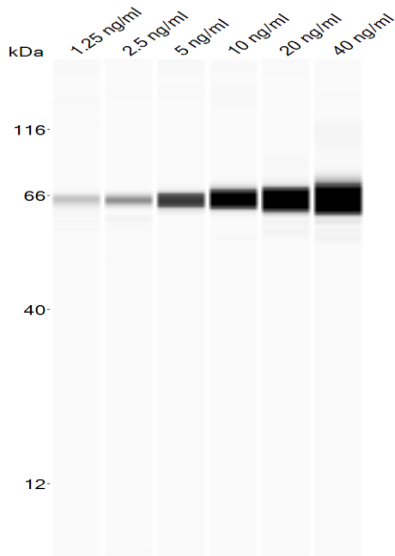


A)



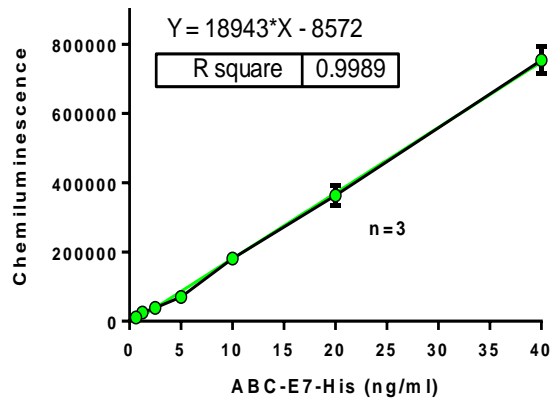
B)

**Example 1. Time kinetics of anti-IgM induced protein phosphorylation in Raji B lymphoma.** Log phase growing Raji cells were stimulated with anti-IgM for 0-30 min. Cell lysates were prepared for simple western analysis of ERK/MAPR phosphorylation A) Electropherogram view. B) Traditional western blot view.



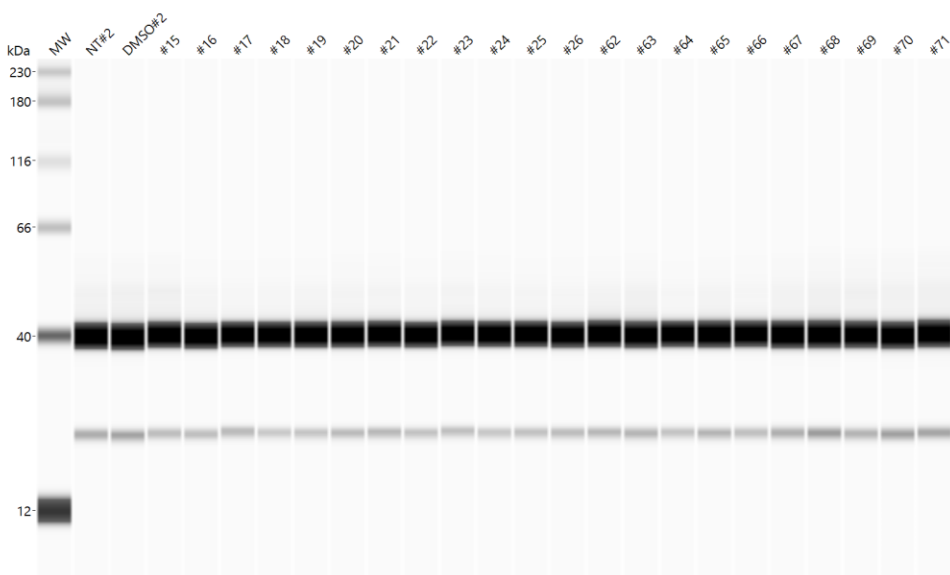
A)

**Linear curve for ABC-E7-His probed with anti-ABC**

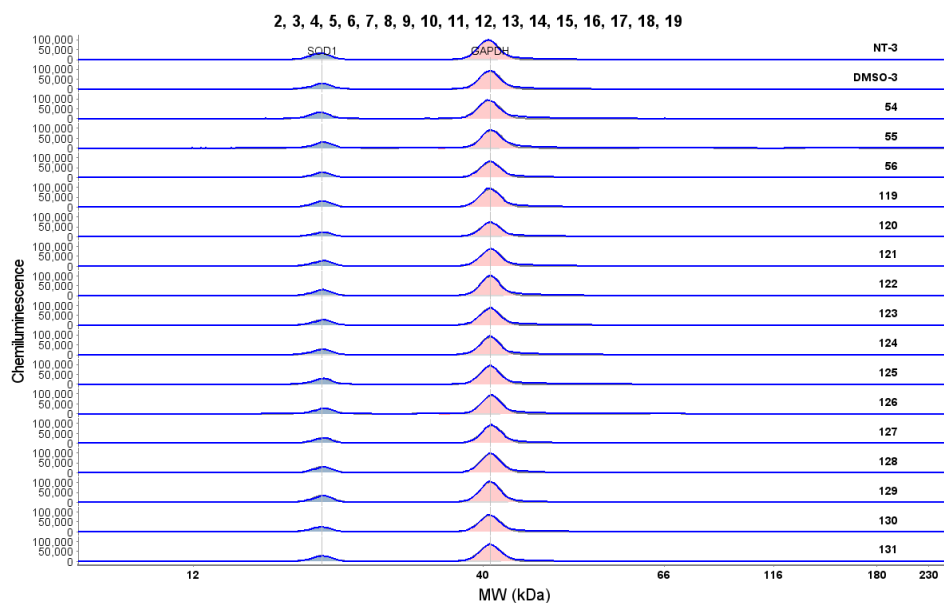


B)

**Example 2. Titration of ABC-E7-his and detection with anti-ABC antibody.** A) Traditional western blot view; B) Linear regression of standard curve for absolute quantitation.



A)



B)

**Example 3. Screening of PROTAC candidates.** Cancer cells were treated with test compounds and then cell lysates were prepared for simple western. A) Traditional western blot view to show the bands for GAPDH (40 KDa) and Target of Interest (22 KDa). B) Electropherogram to show to peaks at 22 and 40 KDa for quantitation of proteins. GAPDH was used as loading control.