Simple Western Assay (Automatic Western Blot) Service

HIGH SENSITIVITY AND REPRODUCIBILITY

ABOUT SIMPLE WESTERN

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

APPLICATIONS

- Absolute or relative quantitation of any protein of interest
- Quick assay development for novel antigens /targets/antibodies
- Clear separation of proteins based on either sizes (MW, 2-440 KDa) or electric charges (pI)
- Reliable quantitation of multiple target proteins / biomarkers in samples of limited amount, including fine needle aspirates, laser capture microdissection, cell culture, etc, from animals or human patients
- 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 marrows and sorted cells
- Discovery and validation of biomarkers to support developing predictive and companion diagnostics
- Characterization of monoclonal and polyclonal antibody binding affinities and specificity
- Characterization of relative specificity of antibody to the target protein of interest
- Quantitation of protein phosphorylation even without phospho-specific antibodies
- Analysis of post-translational protein modifications
- 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 degradation, expression or modifications of any protein or signaling molecule

SAMPLE REQUIREMENTS

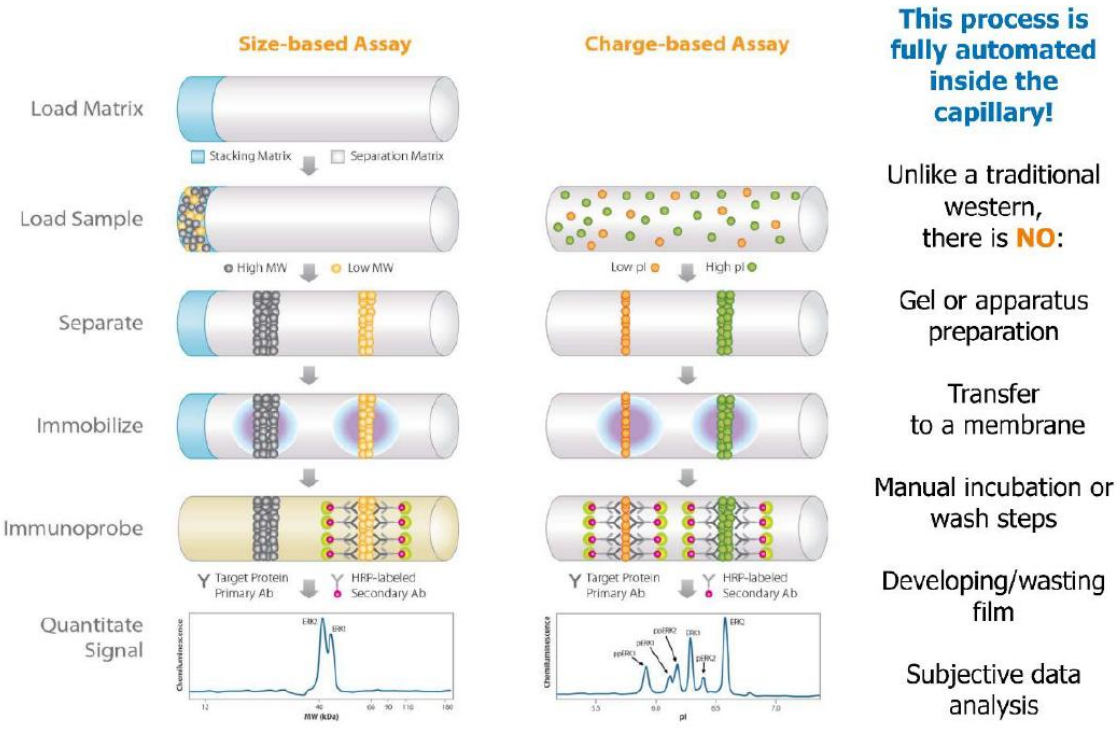
- Samples should be BSL- I or II
- Samples (about > 10 µl of 2 - 4 mg/ml protein) should be submitted
- Any extraction procedures are the responsibility of the researchers. Special arrangements can be made in advance
- Submit samples in a minimum-binding microfuge tubes, accompanied by a template that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

SERVICE FEATURES

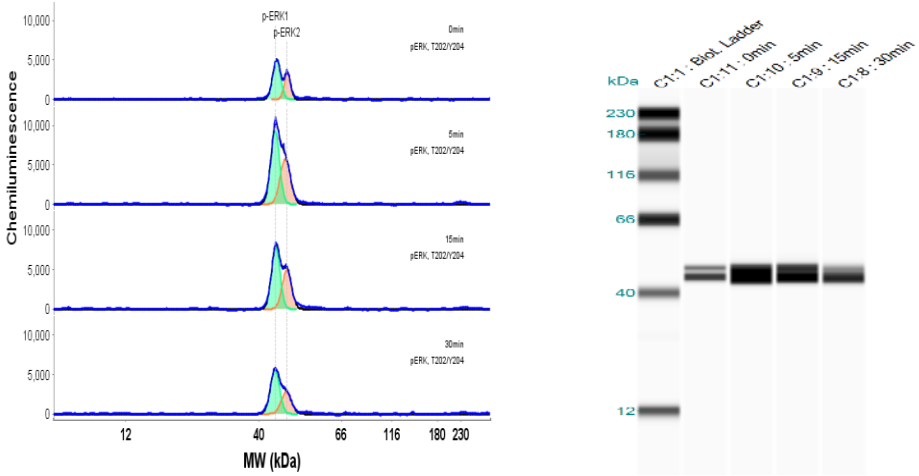
- ❖ Highly sensitive and Automatic Western blot to examine proteins in a minimal amount of sample
- ❖ Multiplex
- ❖ Qualitative and quantitative
- ❖ Highly reproducible

- ❖ 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 cells from 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, Jess, Peggy
- ❖ Timely data delivery: 1-4 weeks or sooner dependent 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 >4000 validated antibodies to examine target proteins*
- ✓ *We offer ~100 human cancer cell lines and various primary human cells for testing drugs and biologicals for specific projects*
- ✓ *We can do homogenization and preparation of tissue lysates*
- ✓ *We can perform protein concentration measurement / normalization using assays such as BCA*

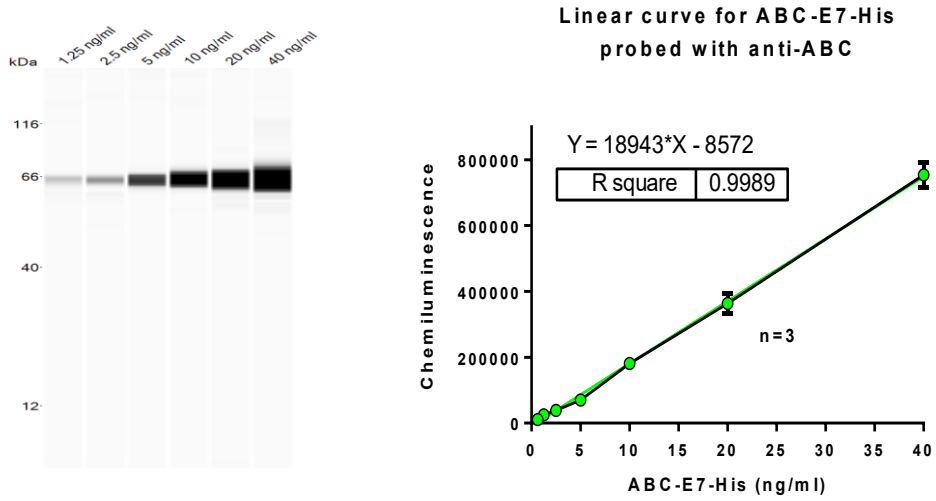
HOW DOES SIMPLEWESTERN WORK



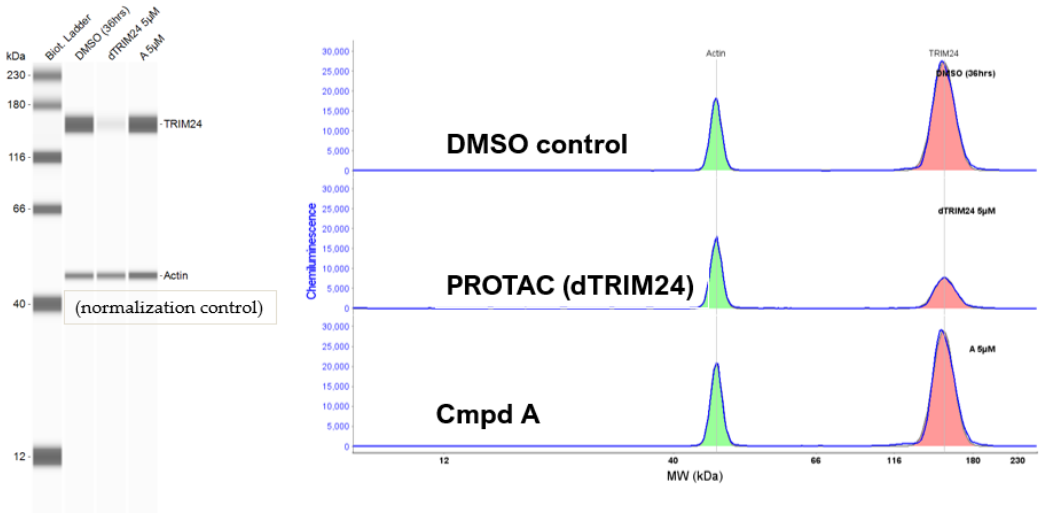
DATA EXAMPLES



Example 1. Time kinetics of anti-IgM induced protein phosphorylation in Raji B lymphoma. Cells stimulated with anti-IgM for 0, 5, 15, and 30 min were lysated and prepared for Simple Western analysis of ERK/MAPR phosphorylation: Electropherogram view (left) and lane view (right).



Example 2. Titration of ABC-E7-his and detection with anti-ABC antibody. Simple Western Lane view (left) and linear regression of standard curve for absolute quantitation (right).



Example 3. Screening of PROTAC candidates. MCF-7 cancer cells were treated with test compounds and then cell lysates were analyzed on Simple Western. Electropherograms showing the bands (left) and peaks (right) of b-actin and TRIM24 in a multiplex assay. b-actin was used as loading control

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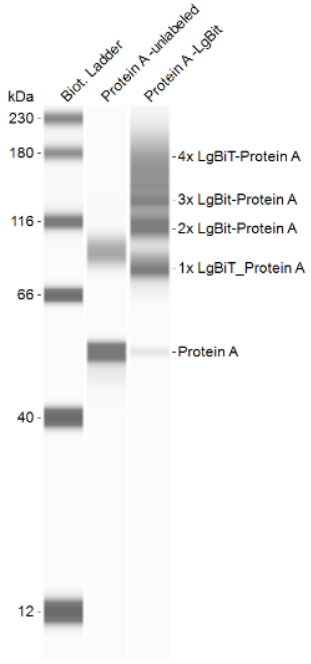
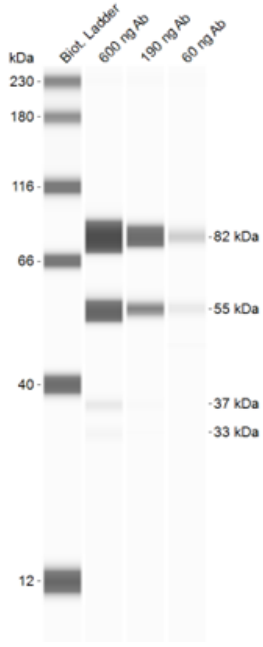


Example 4. Detection of bio-conjugation. Protein A was conjugated with LgBit. Lane view showing the successful conjugation (higher MW bands on the right lane) Non-reducing conditions.



Example 5. Analysis of antibody fragments using total protein assay and non-reducing conditions