Capillary Electrophoresis for Upstream and Downstream Biopharmaceutical Development

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Abstract

In order to bring an antibody or other biologic drug to market, each step of the process needs to be carefully monitored. Charge heterogeneity and apparent molecular weight (MW) via SDS-PAGE are two of the most commonly assessed parameters. Ideally, similar methods should be used from the beginning of development (low expression, complex matrix) to late in production and QC (high concentration, pure material). Capillary techniques, such as iCE and CE-SDS, are currently used heavily in downstream product development but are best suited to purified and higher concentration samples. Here, we present the Simple Western that combines in one novel instrument, Peggy, capillary electrophoresis with an immunoassay to provide highly reproducible and fully automated analysis of monoclonal antibodies. This sensitive technology measures either size or charge in complex samples and provides critical charge heterogeneity, size, and product titer information without the need for sample purification. Data will be presented demonstrating the application of the Simple Western technique and the iCE technology for the analysis of monoclonal antibodies against VEGF and the ability of the two techniques to provide consistency of data across the whole range of product development. In addition we show examples for the unique capability of this technology to assess affinity information for these anti VEGF antibodies to different charge isoforms of VEGF.

Conclusions

- The Simple Western allows for both charge and size based analysis of proteins and monoclonal antibodies (mAb) at low concentrations and in complex biological matrices.
- Analysis of mAb charge heterogeneity is rapidly, reproducibly and quantitatively carried out with Peggy and Simple Western analysis
- The combination of Simple Western and iCE provides a comprehensive analytical solution for complete coverage of biologics development.

Assay Principals

Peggy is a bench top instrument capable of separating proteins by either their size or their charge (Figure 1). The iCE3 provides rapid, high resolution and robust analysis of protein charge heterogeneity (Figure 2). Both platforms utilize capillary electrophoresis as the method of separation. With Peggy, she runs 12 samples simultaneously and up to 96 samples in a single experiment. Samples for Simple Western assays are either denatured (for size separation) or maintained in a native state (for charge separation). Samples are then loaded into capillaries, separated by either size or charge and immobilized to the capillary wall via a proprietary UV capture method. Target proteins are immunoprobed with an antibody followed by HRP-amplified chemiluminescent detection. Peggy fully automates the entire Western blot procedure which results in increased reproducibility and significant time savings. The iCE3 system performs imaged cIEF with direct detection at 280 nm.

Simple Western Assay Principals

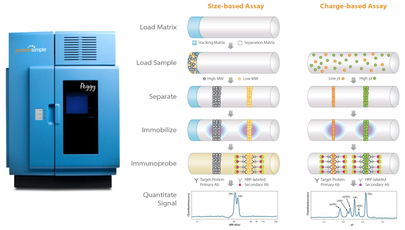
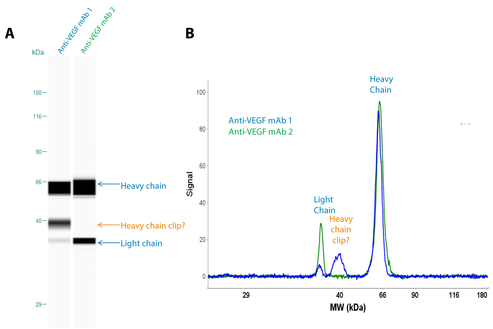


Figure 1: Peggy and steps of a Simple Western assay.

iCE Assay Principals

Figure 2: iCE3 and steps of an iCE assay.

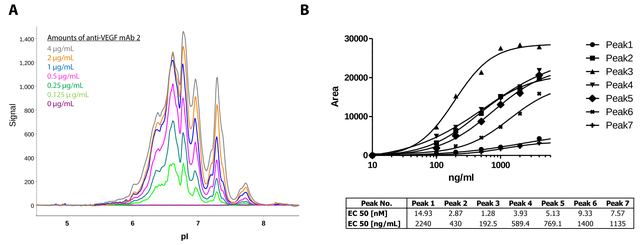
Antibody Integrity Detection



Lane View Electropherogram

Figure 3: Size separation and detection performed on Peggy can be used to characterize antibody integrity. Two different preparations of anti-VEGF mAb were separated in the size mode and detected using an Anti-Goat-Anti-Mouse Secondary HRP Conjugate (ProteinSimple) and chemiluminescent substrate. The presence of an intermediate band/peak between the light and heavy chains was detected in the Simple Western size assay. The data suggests this is a heavy chain clip or an incomplete translated product.

Charge Variant Characterization



Peak No.	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
EC 50 [nM]	14.93	2.87	1.28	3.93	5.13	9.33	7.57
EC 50 [ng/mL]	2240	430	192.5	589.4	769.1	1400	1135

Figure 4: A unique capability of Peggy Simple Western charge separation is the ability to separate different charge variants of a target protein and separately establish the relative affinity of a detection antibody against each of the charge variants. Shown is the charge separation profile of human VEGF-165 (Cell Signaling Technology, p/n 8065SF) at 1 µg/mL probed with mouse monoclonal anti-VEGF antibody (Calbiochem, p/n GF25) at the concentrations indicated in the graph. Detection was performed using the ProteinSimple Goat-Anti-Mouse secondary HRP conjugate. These results show not only the number of charge variants in a VEGF preparation, but also the differential affinity of the antibody against the different charge variants. (A) shows the electropherograms in response to different antibody concentrations. (B) shows the affinity curves of the results generated in (A).

iCE and Simple Western Provide Equivalent Results

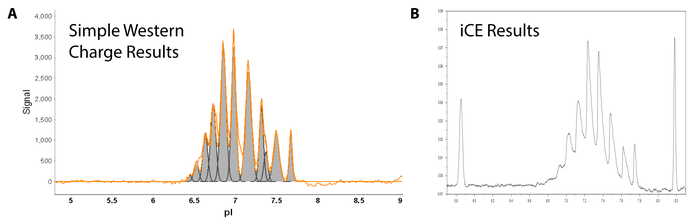


Figure 5: Charge profiles of VEGF mAb separated on either Peggy (Simple Western Charge assay) and iCE are equivalent in terms of calculated pI and peak areas. The same preparation of VEGF mAb was separated on either Peggy (Charge mode) or the iCE3. 1.2 µg/mL of VEGF antibody was run in the Simple Western charge assay and 0.5 mg/mL of VEGF antibody (buffer exchanged in 20 mM Tris pH 8.8) was run on the iCE3.

Detecting Post-translational Modifications

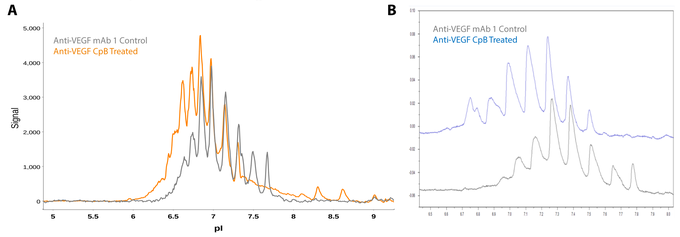


Figure 6: Similar and equivalent charge profiles observed with Peggy and iCE3 are obtained when analyzing and detecting post-translational modifications. VEGF mAb was treated with 1 unit of carboxypeptidase (CpB) for one hour at 37°C and then evaluated on either Peggy (Simple Western charge assay) or the iCE 3. A clear shift in the charge profile is observed with CpB treatment and the charge profile and peak areas identified are similar between Peggy and the iCE 3.

Complementary Coverage of Biologics Development

Figure 7: Peggy and the Simple Western is the ideal platform for separating analytes by either charge or size in unpurified as well as low concentration samples during the early phases of biologics development. The iCE system with accurate and easy quantitation of charge isoforms is the ideal platform later stages of product development and QC.