FFE Service GmbH

Novel iZE Separation Of Monoclonal Antibody Isoforms By Free Flow Electrophoresis

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CE in the BIOTECHNOLOGY & PHARMACEUTICAL Industries

Introduction

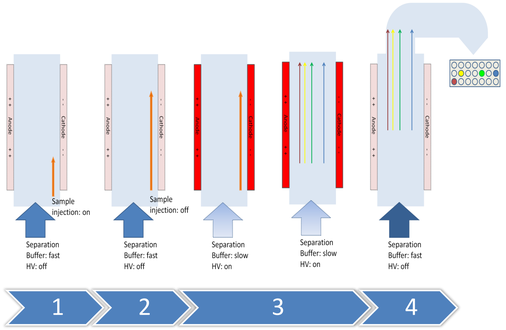
Many clinically and scientifically relevant proteins undergo posttranslational modification (PTM) stemming for example from chemical adducts (glycosylation, phosphorylation, etc.) or mRNA splicing. These protein isoforms can differ from each other in their activity and therefore may need to be closely studied. For therapeutic and clinical applications, it may be beneficial and/or necessary to separate isoforms from each other ahead of clinical application. **Monoclonal antibodies (mABs)** are a good example of such proteins, often bearing alternative and closely related PTMs that make the mAB isoforms challenging to separate, but where separation can be important for characterization and ultimately for commercial production.

The matrix-free separation of protein isoforms by Free Flow Electrophoresis (FFE) is an ideal tool for **preparative separation of protein isoforms**. FFE's unique attributes include fast separation, high sample-throughput and ability to recover sample and not merely analyze them.

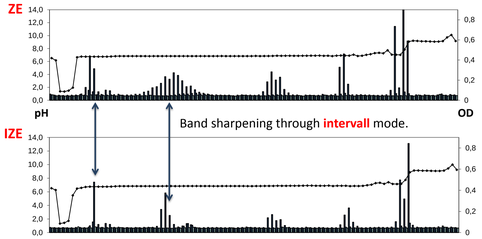
In a novel approach, mAB isoforms were separated by FFE using the newly developed interval zone electrophoresis (iZE) mode. The buffers for iZE contain only well-defined and relatively **inexpensive chemicals** (one acid, one base and mannitol), prepared as a stepwise pH gradient in the FFE separation chamber. The iZE mode results in exquisitely sharp separation of the very closely related PTM isoforms of mABs.

The iZE mode provides additional benefits beyond **very high separation resolution**. Specifically, iZE was developed to **eliminate the use of polymers and ampholytes**, making it directly compatible with downstream analytical techniques (e.g. crystallography, mass spec, etc.), and facilitating use in preclinical or clinical applications by avoiding complicated post-separation clean-up procedures.

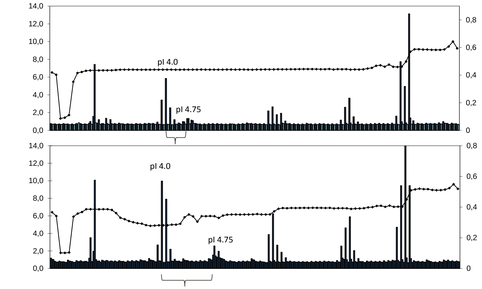
Principle of IZE Free Flow Electrophoresis



Comparison: ZE vs. IZE



Comparison: IZE constant pH vs. pH steps



Separation of mAB by FFE-IZE

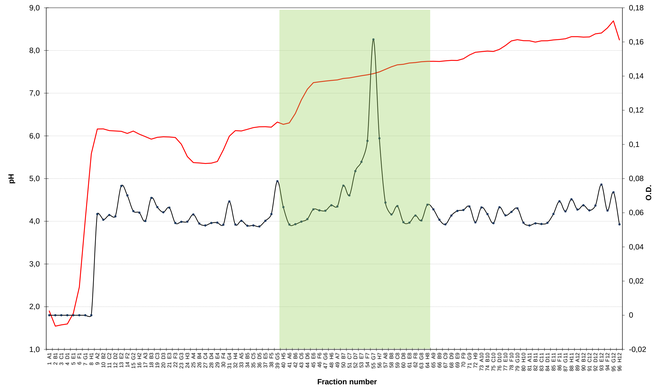


Figure 1: UV-profile (black line) of the separated mAB sample and pH profile (red line). The fractions within the green area were applied to the IEF gel (Figure 2).

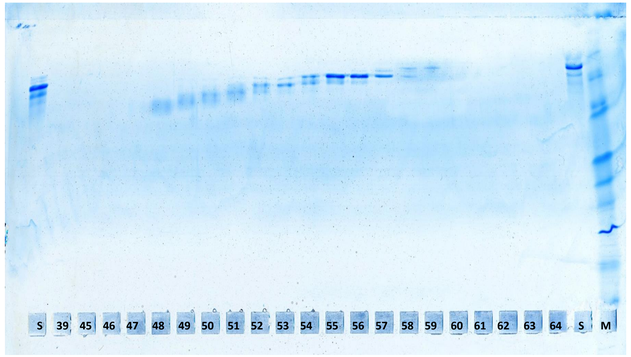
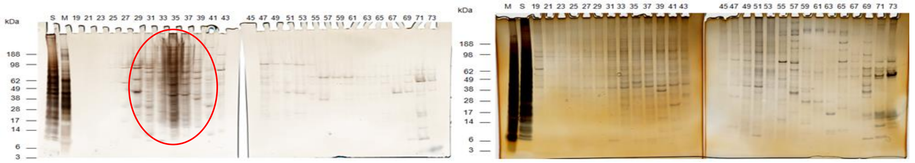


Figure 2: IEF-PAGE of the crude sample (S) and selected fractions of IZE FFE separated mAB sample. M: Serva IEF Marker.

Comparison: FFE-IEF vs. IZE-pH (cell lysate)



Material and Methods

Protein throughput:	60 µg/interval (max. 100 µg/interval)
Voltage:	1400 V (max. 1800 V)
Current	75 mA
Interval/Separation time :	10 minutes (min. 4 minutes)
Temperature:	10 °C (min. 5 °C)
IEF-gels	Serva Focus gel pH 6-11

The Antibody samples for this study were supplied by Biogen, RTP, North Carolina, USA.

Protein isoform separation using FFE:

- **Entirely liquid** methods (no gels, no matrix)
- **Native conditions** maintain structure and function of separated isoforms
- **Quick and easy** to set up and operate
- **High resolution** separations
- Scale **from analytical to milligram** preparation ability
- One instrument supports **many different separation needs**:
  - Flexible and adaptable methods
  - From simple to highly complex samples
  - Specific protein targets (and their unique characteristics)
  - From basic research to production-scale throughput

Traditional IEF method:

- The method of choice for
  - Characterizing complex mixtures
  - Screening preparations of unknown isoelectric composition
- Uses ampholyte cocktails
  - Commercially available in a variety of defined pH ranges, from broad to narrow
- Unique continuous run method (not batch) can allow high separation capacity

New iZE method:

- The method of choice for
  - Achieving exquisitely fine resolution
  - Separating targets with known isoelectric properties
- Unique benefits versus IEF
  - Easily tuned for precise pH ranges using same reagents
  - Reduces precipitation concern: protein is never held at its isoelectric point
  - Reduced chemical complexity of separation media
  - Improved compatibility with downstream methods
  - Reduced cost per run