



# cIEF in Early Discovery, Product Quality and Stability Assessment

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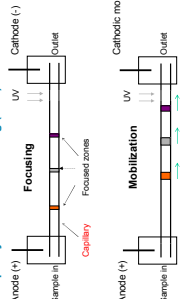
## Abstract

There are many sources of charge heterogeneity in an antibody. Methods usually used to monitor the charge heterogeneity of an antibody include ion exchange chromatography, IEF gel, capillary isoelectric focusing (cIEF), and imaged cIEF. Imaged cIEF eliminates the mobilization step in cIEF, and provides faster run time and method development. cIEF technique has been successfully used in early discovery, product quality and stability assessment. Examples of cIEF used in early discovery program proved that imaged cIEF and WCX-10 fractions showed that some of the WCX-10 acidic fractions could be caused by conformational differences. cIEF analysis of stability samples demonstrated advantages in resolution and quantitation over weak cation exchange chromatography.

## Methods and materials

Beckman Proteomelab PA800 capillary electrophoresis system  
Convergen biosciences ICE 280 system  
Dionex ProPac WCX-10 analytical column and Shimadzu HPLC system  
In-house antibodies: AB-1, AB-2, and AB-3

## Capillary isoelectric focusing (cIEF)

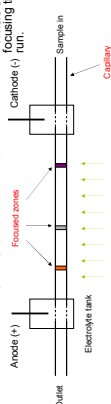


- cIEF on Beckman PA800 uses single-point UV detection.
- cIEF process includes two steps: focusing and mobilization.

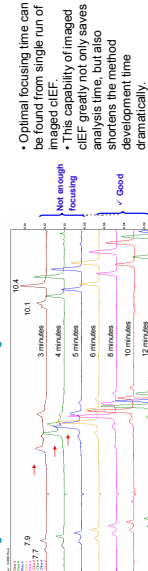
## Imaged cIEF

- Imaged cIEF eliminates mobilization process – faster run and higher resolution.

- Whole-column detection – optimal measuring time can be found from single run.

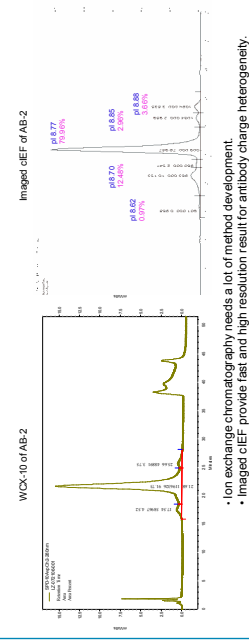


## Imaged cIEF of AB-1-different focusing time

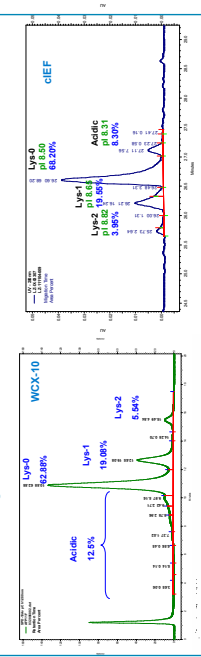


- Optimal focusing time can be found from single run of imaged cIEF. Quality of imaged cIEF greatly not only saves analysis time, but also shortens the method development time dramatically.

## Imaged cIEF is a powerful tool in early discovery

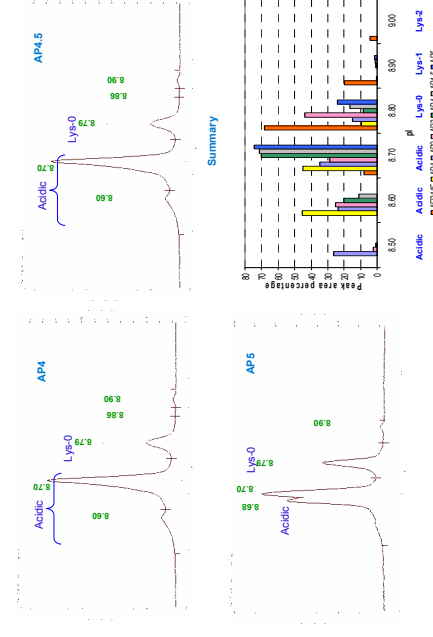


## WCX-10, cIEF, and Imaged cIEF of AB-1



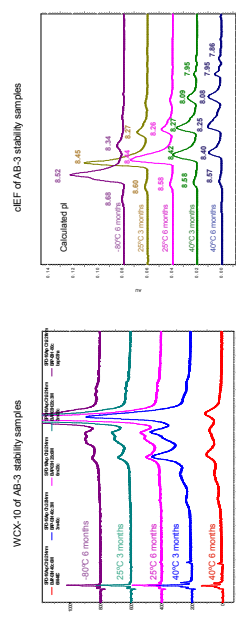
- cIEF and imaged cIEF showed less amount of acidic species than WCX-10.

## Imaged cIEF of AB-1 WCX-10 fine fractions (continued)



- All WCX-10 acidic fine fractions were run on cIEF and imaged cIEF with 6M urea. The addition of 6M urea didn't change the peak profiles compare to those without urea.
- cIEF and imaged cIEF results were very comparable.
- None of the WCX-10 acidic fractions was pure in cIEF.
- WCX-10 acidic fractions all had some pI ~8.8 (Lys-0) species.
- Some of the WCX-10 acidic fractions could be caused by conformational differences.

## cIEF provides high resolution for stability assessment



- WCX-10 of stability samples showed acidic shift.
- Limited resolution made quantitation difficult.
- cIEF showed higher resolution, easy for quantitation.

## Conclusions

cIEF and imaged cIEF was used successfully in early discovery, product quality and stability assessment. Compared to weak cation exchange chromatography, cIEF has the advantages of minimum interaction with the capillary, enhanced resolution, low sample consumption, fast analysis, and fast method development (imaged cIEF).



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