

# ***AESlyte™ Carrier Ampholytes***

## ***Technical Note***

# AESlyte™

## for cIEF/icIEF

Carrier ampholytes (CAs) are complex mixtures of small (200 to 1,200 Da) amphoteric molecules that are good carriers of conductivity and buffering capacity at their respective isoelectric point (pI) 1. AESlyte™ carrier ampholytes (CAs) are industrial next generation CAs, developed by Advanced Electrophoresis Solutions Ltd, Canada. Our comprehensive CAs are the only solution in the market to support biopharma's entire method lifecycle for commercial products, such as mAbs, ADCs, bispecifics, fusion proteins, enzymes etc.

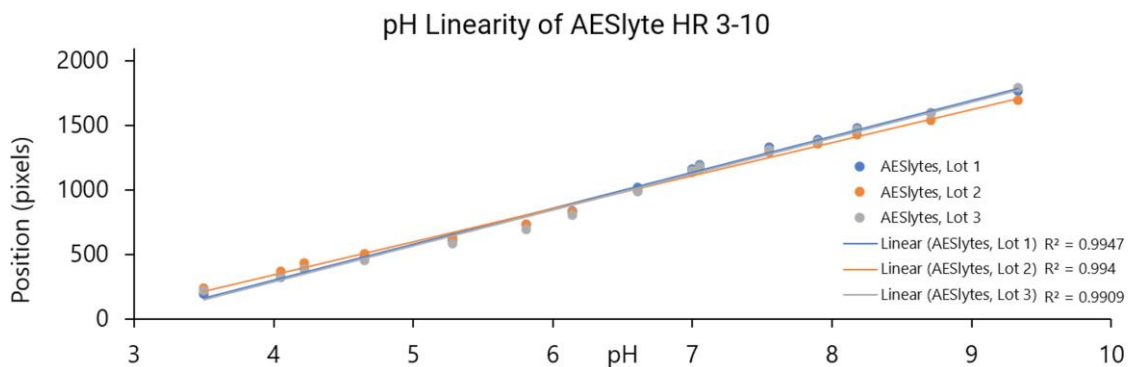


Fig.1 a) pH gradient linearity of AESlyte™ HR 3-10

# Good lot to lot consistency

The separation resolution and reproducibility of icIEF have been greatly affected by different sources or lot of carrier ampholytes. Traditional synthesis and production of carrier ampholytes lack precise control for icIEF applications, especially for complex biologics. SOPs of icIEF methods developed cannot be applied when desired icIEF profile of protein charge variants could not be obtained due to the lack of icIEF performance control of old CAs. This causes severe issues on commercial product release and sale.

- *Extremely stable*
- *High buffer capacity*
- *Low UV 280 nm absorbance*
- *Low protein interaction*
- *Lot to lot pI variation  $\pm 0.3$*
- *Narrow range ampholytes suitable for CEInfinite icIEF preparation*

AES supported several major biopharma companies and developed suitable CAs based on AES' proprietary manufacturing process and quality control. AES is able to provide next gen CAs with consistent icIEF performance to meet client's SOPs of icIEF, and became their critical reagent supplier. Beside the good pH gradient linearity and lot to lot consistency, shown in figure 1, AESlyte™ has advantages:

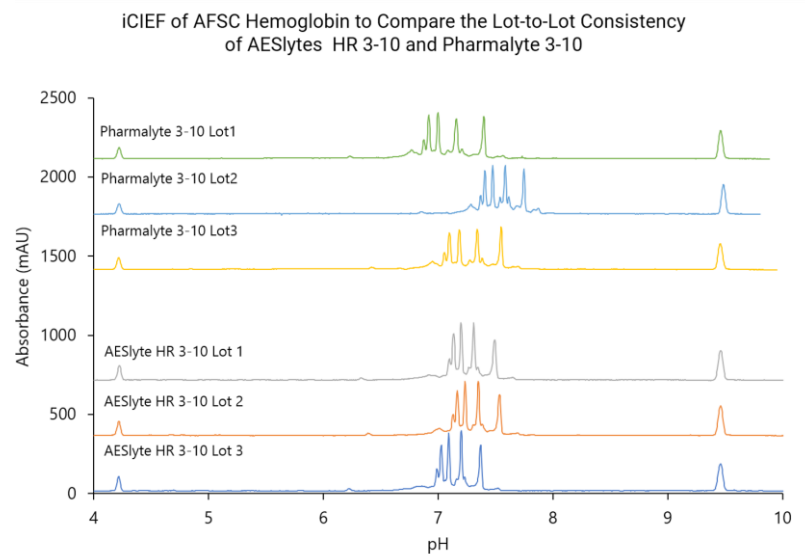


Fig.1 b) Pharmalyte 3-10 three lots and AESlyte™ HR3-10 three lots comparison.

# AESlyte™ HR/SH/UH series

## HR series

High resolution (HR), broad and narrow range carrier ampholytes, for cIEF and icIEF of mAbs and ADCs, bispecific. Suitable for CEInfinite preparative system for icIEF charge variant fraction and collection.

New high pH range AESlyte HR 9-12 was recently released. Cytochrome C (bovine) with pI range of 10.3 – 10.8 was ran with following conditions, and result shown in figure.2.

### Sample preparation:

- 35  $\mu\text{L}$  of 1 % Methyl cellulose
- 4  $\mu\text{L}$  of AESlyte HR 9-12
- 4  $\mu\text{L}$  of 500 mM L-arginine
- 0.5  $\mu\text{L}$  of each marker (pI 9.46, 11.20)
- 1.25  $\mu\text{L}$  of bovine Cytochrome C (0.25mg/ml)
- 54.25  $\mu\text{L}$  of Nanopure H<sub>2</sub>O

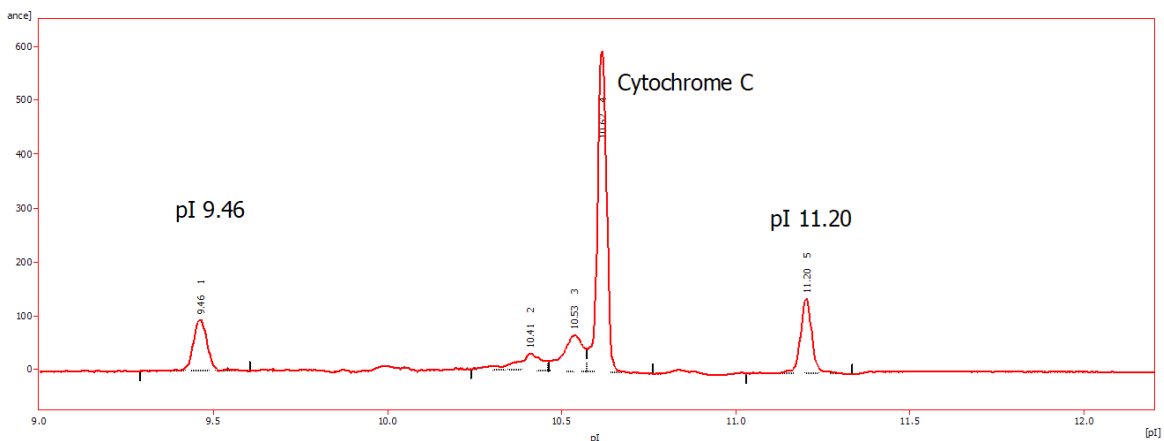


Fig.2 Cytochrome C icIEF e-gram with AESlyte™ HR 9-12

# AESlyte™ HR/SH/UH series

## SH series

Super High (SH) has unique functional groups which improve iCIEF resolution for mAbs when mixed with AESlyte™ HR series. Works good for mAbs and ADCs, bispecific etc.

Figure 3 below demonstrate iCIEF separations with very high resolution and reproducibility using commercial antibody drug conjugate (ADCs) and monoclonal antibodies (mAbs). Samples were ran in CEInfinite system using AESlyte™ HR/SH series.

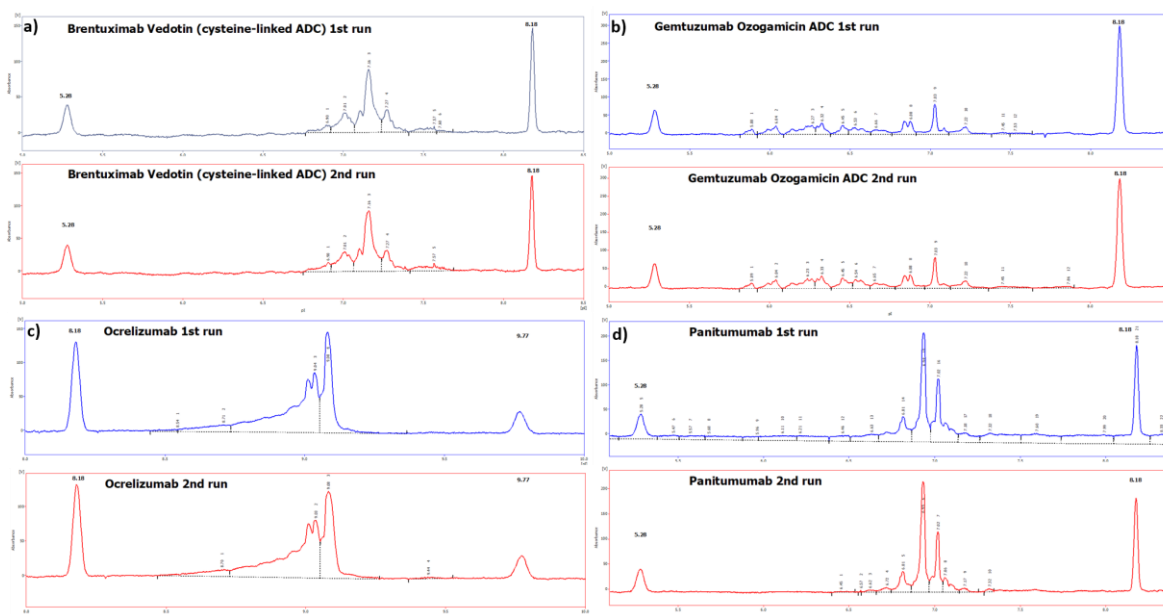


Fig.3 Commercial antibody drug conjugate (ADCs) and monoclonal antibodies (mAbs) ran with CEInfinite system and AESlyte HR/SH series.

a) Brentuximab Vedotin (cysteine-linked ADC) 0.14mg/ml HR 3-10

b) Gemtuzumab Ozogamicin ADC 0.14mg/ml, SH 3-10

c) Ocrelizumab 0.24 mg/ml, HR 3-10/HR 8-10.5 d) Panitumumab 0.2mg/ml, HR 3-10/HR 5-8.

# AESlyte™ HR/SH/UH series

## UH series

Ultra High (UH) carrier ampholytes, optimized for complex proteins such as fusion proteins and enzymes.

Commercial fusion protein Etanercept 0.5mg/ml, 3 runs in CEInfinite analytical system with AESlyte UH 3-10 with pI marker 4.22 and 8.40, CVs consistently less than 4%, shown in figure.4.

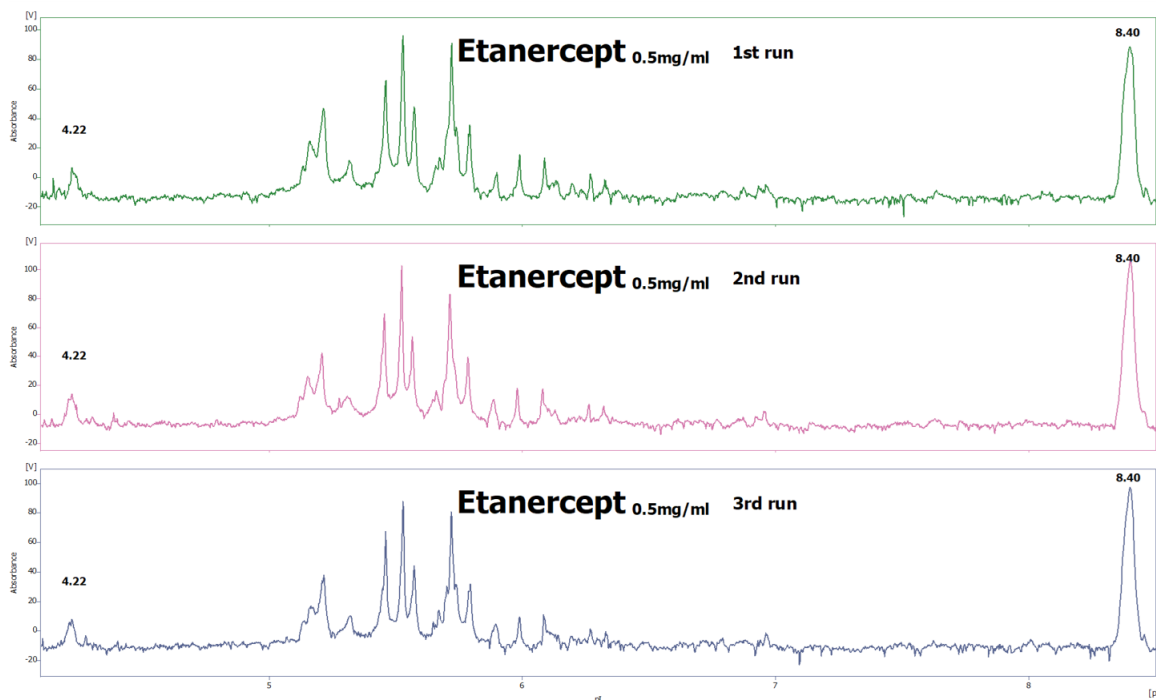


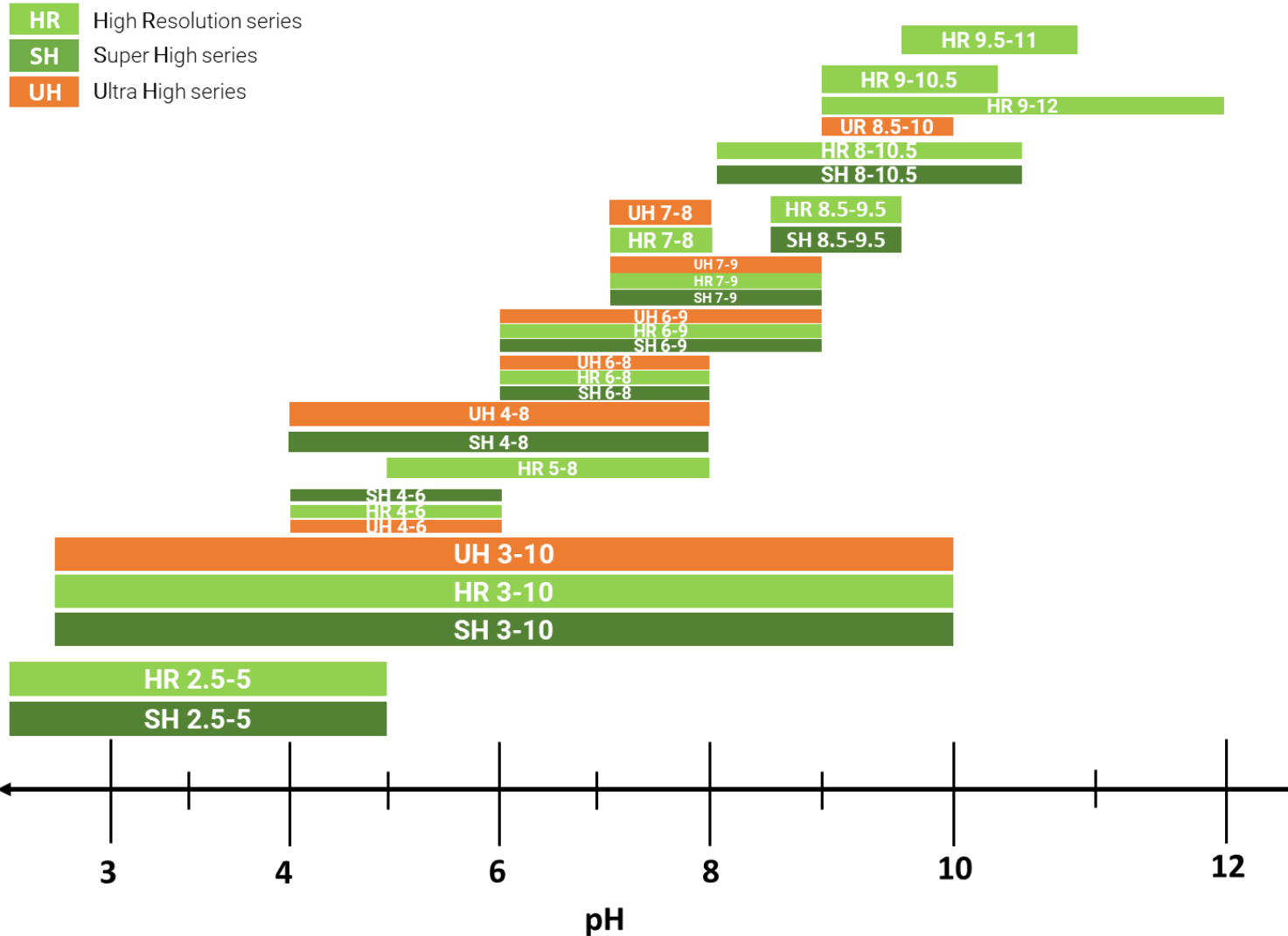
Fig.4 Commercial Etanercept 0.5mg/ml, runs in CEInfinite system with AESlyte™ UH 3-10.

### Reference:

1. Righetti, P.G.; et al. Carrier ampholytes for IEF, on their fortieth anniversary (1967-2007), brought to trial in court: The verdict., *Electrophoresis* 2007, 28,3799–3810.

2. Christian Wenz, Agilent Technologies, Inc. Comparison of Different Brands of Carrier Ampholytes for Monoclonal Antibody Charge Heterogeneity Analysis by Capillary Isoelectric Focusing.

# Product line of AESlyte™



# Your iCIEF Total Solution Provider

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AES critical reagent service brings  
your QA/QC to another level.  
AES has the expertise to do :

- *Carrier ampholyte customizations*
  - *Existing molecule iCIEF profile matching*
  - *Method development for fusion protein  
or other complex molecules*
  - *Guaranteed lot to lot consistency*
  - *Re-supply service*
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