

Biotherapeutic purification example: single chain variable fragment

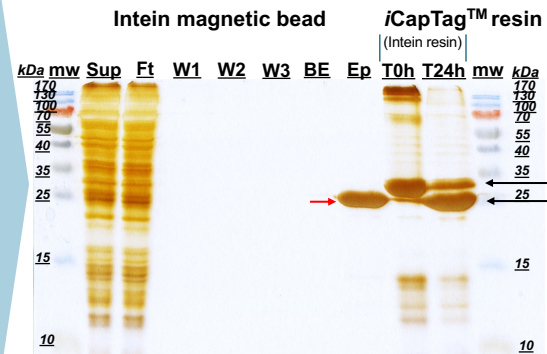
2 mL culture supernatant
Expi293F™ (Gibco)

Yield: ~98.3 µg (3.47 nmol)
per 3.0 x10⁶ mag beads

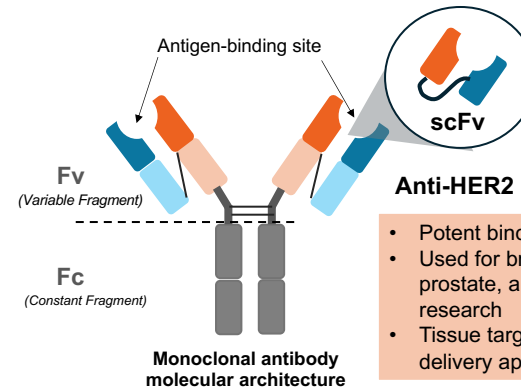
Purity: >99.99%

mw: molecular weight ladder ref.;
Sup: Cell culture supernatant;
Ft: Binding flowthrough;
W1: Washing 1 (pH 8.5);
W2: Washing 2 (pH 8.5);
W3: Washing 3 (Di water);
BE: buffer exchange (10 mM
ammonium acetate at pH 6.2);
Ep: Elution pool
t1-24hr: time point resin samples;
p: Tagged scFv bound to the resin
c: tagless scFv in the mobile phase

Silver stain- purification analysis



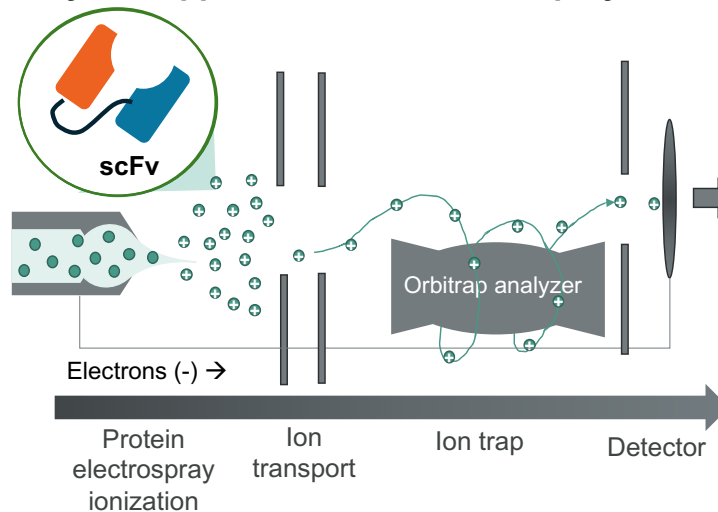
Elution buffer: 10 mM ammonium acetate at pH ~6.2



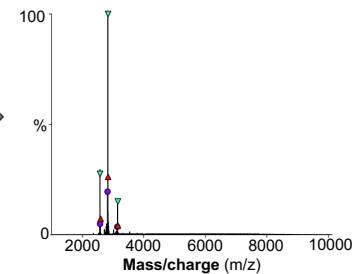
Anti-HER2 ML39 scFv

- Potent binding (~1 nM kD)
- Used for breast, colon, prostate, and lung cancer research
- Tissue targeting drug delivery applications

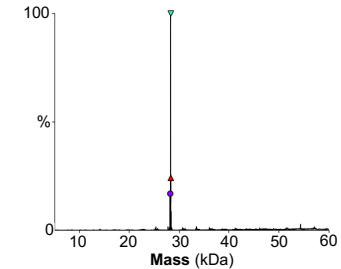
Analytical Application: Nanoelectrospray ionization orbitrap mass spectrometry characterization



Spectra analysis



Theoretical mass:
28,359 Da



Mass (Da)	Intensity	DScore	Name
28,214	12.09	84.13	A
28,351	70.82	90.19	B
28,373	17.10	81.45	C

Native mass spectrometry experiments were performed on a Q Exactive™ UHMR (Ultra-High Mass Range) Hybrid Quadrupole-Orbitrap™ by Josh Gilbert in the Campus Chemical Instrumentation Center Mass Spectrometry and Proteomics Facility and supported by the NIH RM1 Native Mass Spectrometry Guided Structural Biology Center (RM1GM149374).