

MAb/ADC Fragment Separation after IDeS digestion

SEC

HIC

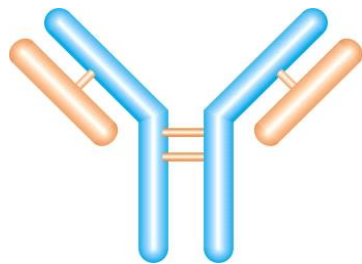
RPLC Proteomix[®] RP-1000



MAb fragments

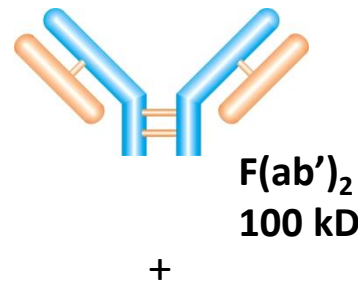
IDeS enzyme digestion

Human IgG1: HTCPPCPAPPELLG↓GPSVF
 Human IgG2: VECPPCPAPP_VA↓GPSVF
 Human IgG3: PPCPRCPAPPELLG↓GPSVF
 Human IgG4: PHAHHAQAPEFLG↓GPSVF

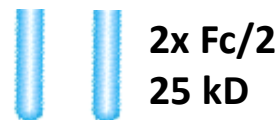


Intact mAb
150 kD

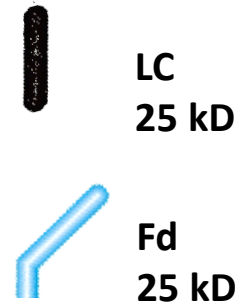
IDeS digestion →



+



DTT ↗



For mAb:
Fc/2, LC, Fd

For ADC:
LC0, LC1, Fc/2, Fd1,
Fd2, Fd3,

Mab and ADC (2 mg/mL each) were incubated with IDeS at 37°C for 1 hour. Digestion buffer: 10 mM phosphate buffer with 150 mM NaCl at pH 6.6.

MAb fragments from IDeS enzyme digestion

ZM1022

SEC Analysis on Zenix SEC-300 (7830)

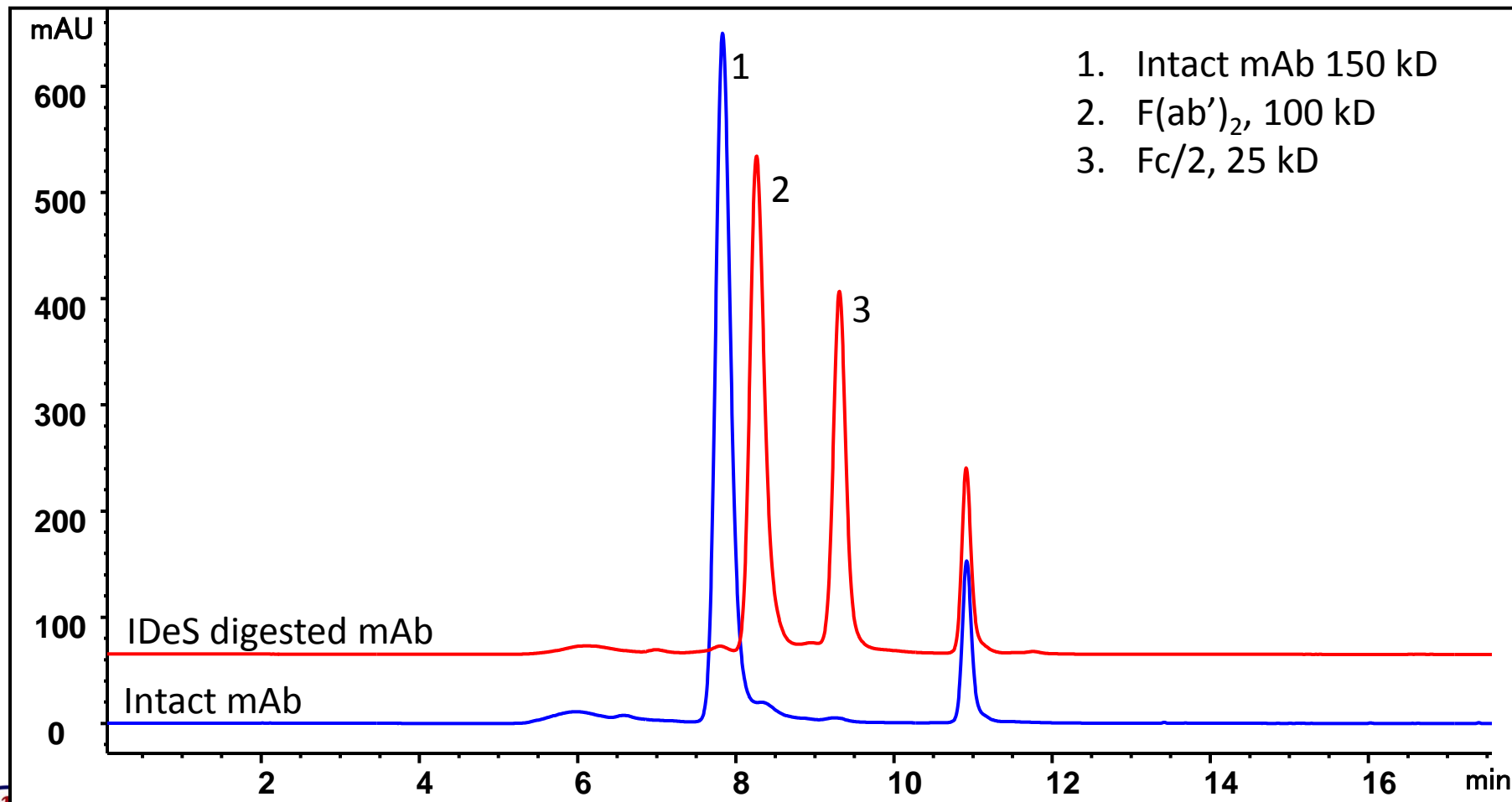
Column: Zenix[®] SEC-300 (3 μm, 300 Å, 7.8 x 300 mm)

Mobile phase: 150 mM Phosphate buffer, pH 7.0

Mab digestion: Mab 5 mg/mL was incubated with FragIT IDeS resin at 37°C for 1 hour

Flow rate: 1 mL/min; Detector: UV 214 nm; Column temperature: Room temperature

Injection: 15 μg IDeS digested mAb and 12 μg undigested mAb



Herceptin and fragment Analysis on Zenix-C SEC-300 (7830)

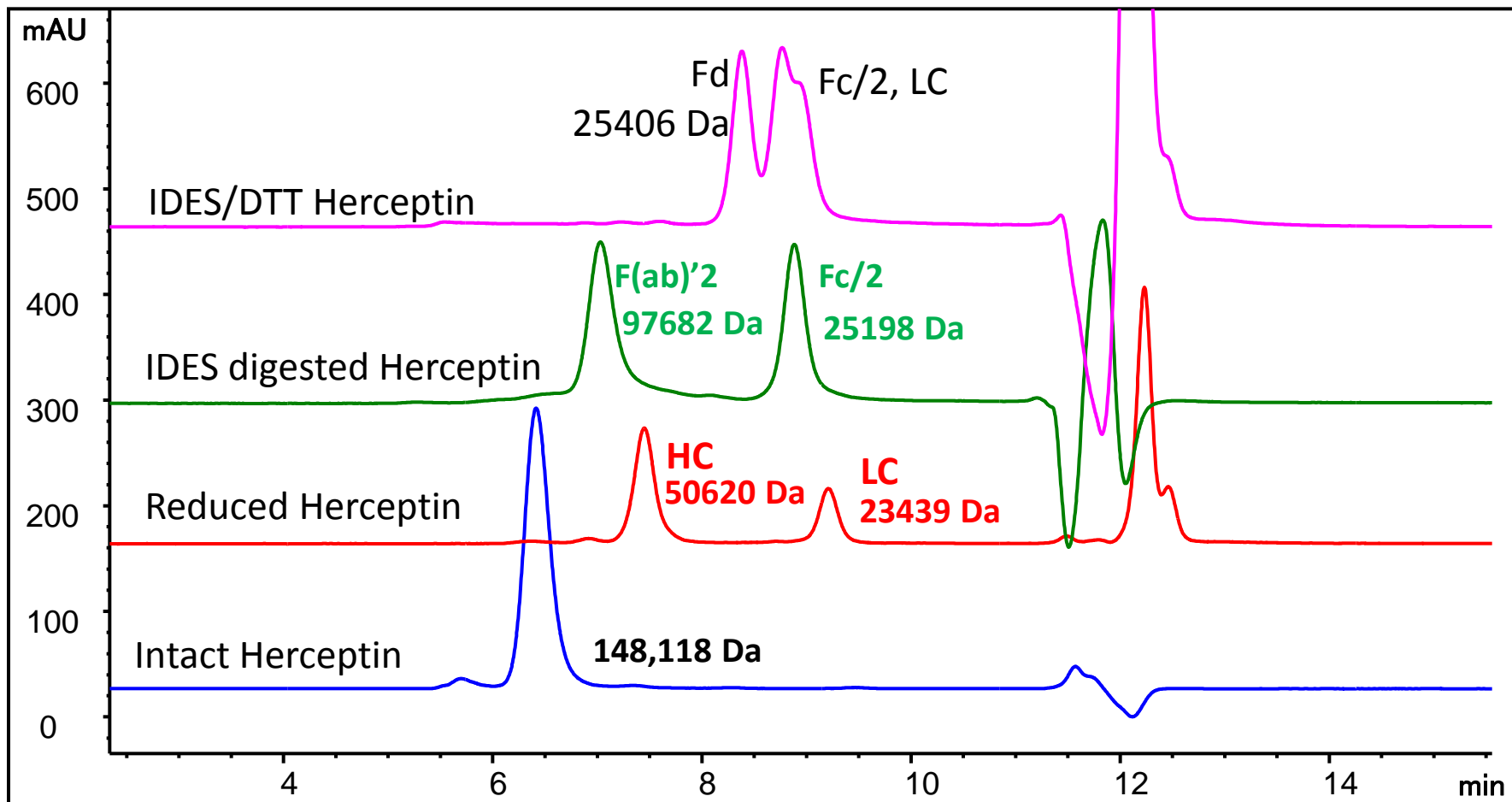
0.1% TFA, 0.1% formic acid and 20% ACN

Column: Zenix[®] - C SEC-300 (3 μ m, 300 \AA , 4.6 x 300 mm)

Mobile phase: 0.1% TFA, 0.1% formic acid and 20% ACN

Flow rate: 0.35 mL/min; Detector: UV 280 nm; Column temperature: 25 $^{\circ}$ C

Injection volume: 2 μ g for each sample

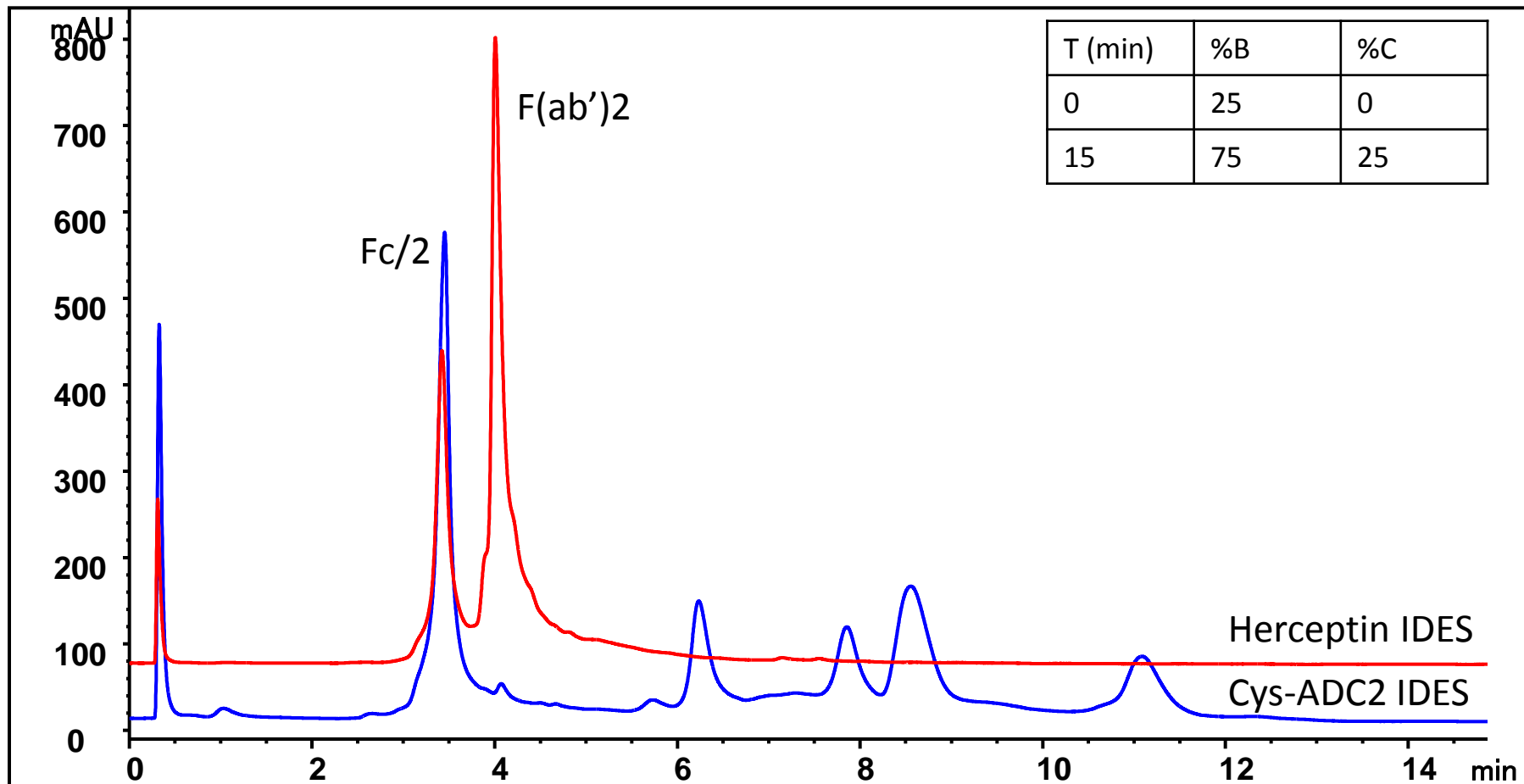


Herceptin and cysteine-ADC fragments after IDES separation on HIC

Column: Proteomix HIC Butyl-NP5 (4.6 x 35 mm)

Flow rate: 0.8 mL/min, Detector: UV 214 nm

Mobile phase: A: 2M ammonium sulfate in 0.025M sodium phosphate, pH 7.0, B: 0.025M sodium phosphate pH 7.0, C : 100%IPA; Column temperature: 25 °C; Injection 10 μ L for herceptin, 20 μ L for cysteine-ADC 1 mg/mL in 750 mM ammonium sulfate 25 mM sodium phosphate pH7.0



mAb/ADC fragment separation on Proteomix® RP-1000

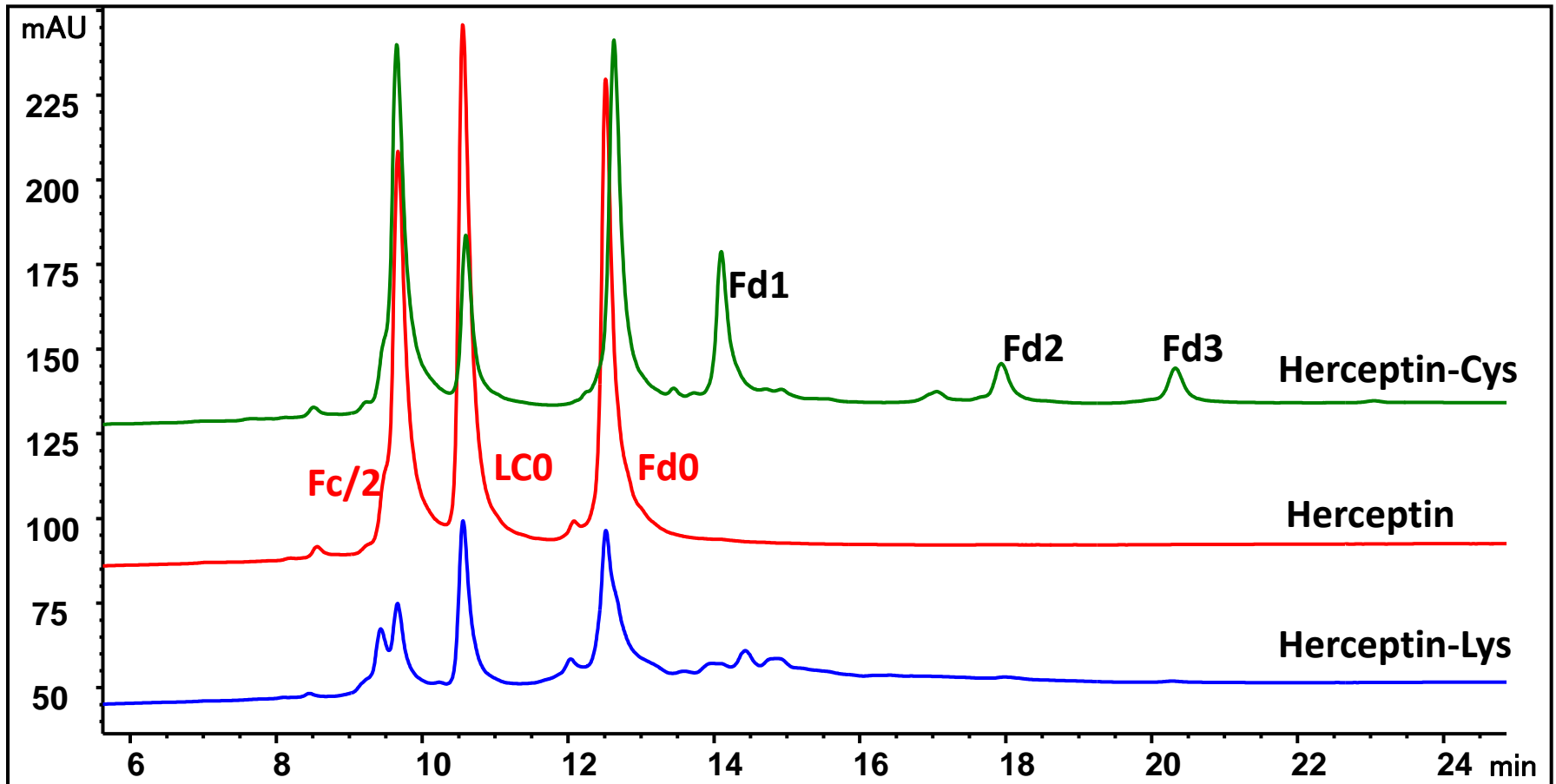
IDeS digested and reduced-Herceptin vs. Herceptin-Lys, Cys

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 0.4 mL/min; Detector: UV 214 nm; Column temperature: 80 °C; Column pressure: 45 bar;

Injection: 3 µL for IDeS digested Herceptin, Lys-ADC and Cys-ADC (1mg/mL each)



After IDeS digestion, 4M guanidine was added and the samples were reduced with 20 mM DTT for 30 min at 56 degree °C.



Product Information

Column	Part number
Zenix [®] SEC-300 (3 μm , 300 \AA , 7.8 x 300 mm)	213300-7830
Zenix [®] -C SEC-300 (3 μm , 300 \AA , 4.6 x 300 mm)	233300-4630
Proteomix [®] HIC Butyl-NP5 (5 μm , NP, 4.6 x 35 mm)	413NP5-4603
Proteomix [®] RP-1000 (5 μm , 1000 \AA , 2.1 x 50 mm)	465950-2105