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Product Information

SigmaMAb Antibody Drug Conjugate (ADC) Mimic
Antibody Cysteine-Fluorophore Conjugate Standard

Catalog Number **MSQC8**
Storage Temperature –20 °C

Product Description

SigmaMAb Antibody Drug Conjugate (ADC) Mimic consists of the human universal monoclonal antibody standard (Catalog Number MSQC4, an IgG1 monoclonal antibody) conjugated to dansyl fluorophores via an LC-SMCC crosslinker (see Figure 1). Each dansyl-LC-SMCC attachment has a total mass of 668 Da. As with commercially-produced cysteine-linked ADCs, reduction of cystine disulfide bridges gives an even number of attachment points (cysteine residues) resulting in a distribution of 0, 2, 4, 6, or 8 dansyl molecules per antibody. The average drug-to-antibody ratio (DAR) as measured by mass spectrometry and hydrophobic interaction chromatography is 4±0.8, similar to commercial cysteine-linked ADCs. Lot-specific DAR values can be found in the Certificate of Analysis on the product display page on the website.

The SigmaMAb ADC Mimic in its native state can be run on a mass spectrometer to give distinct peaks 1,336 Da apart, representing the 0, 2, 4, 6, and 8 distribution of fluorophores per antibody (see Figure 2). The theoretical average masses of various deglycosylated conjugate forms are listed in the Table 1.

Table 1.
Average Masses of Antibody Drug Conjugates

ADC Mimic Form	Deglycosylated Average Mass
Unconjugated mAb	143,769
2 Drug-linker:mAb	145,105
4 Drug-linker:mAb	146,441
6 Drug-linker:mAb	147,777
8 Drug-linker:mAb	149,113

The SigmaMAb ADC Mimic can be reduced to break remaining disulfide bridges, such that the heavy chain will contain 0, 1, 2, or 3 fluorophores per molecule and the light chain will contain 0 or 1 fluorophores, with all species being 668 Da apart (see Figure 3).

Figure 1.
Schematic of SigmaMAb ADC Mimic

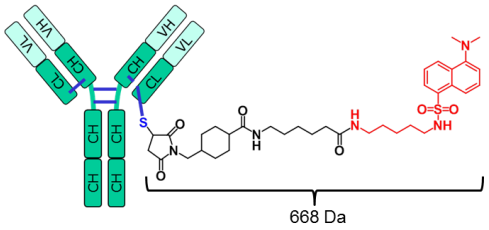
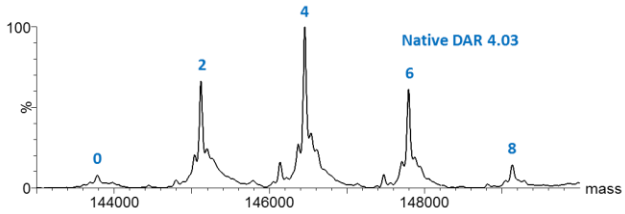
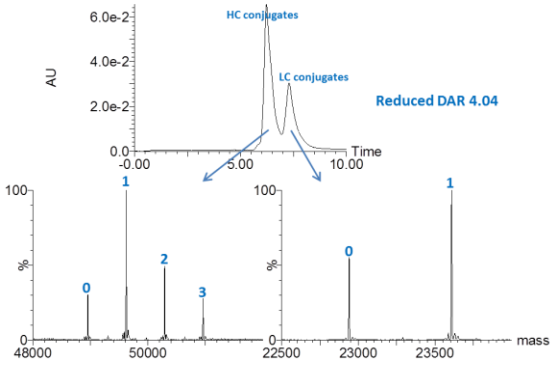


Figure 2.
Native mass spectrum of deglycosylated SigmaMAb ADC Mimic



2 mm × 30 cm TSKgel® SuperSW3000 SEC column
isocratic 70 µL/min, 100 mM $NH_4CH_3CO_2$, pH 7
14 µg injection
Xevo G2 QToF 1000–8000 m/z

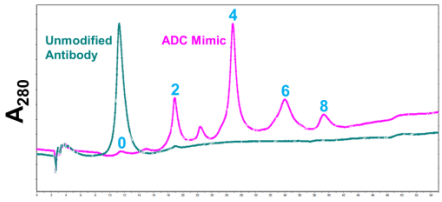
Figure 3.
SEC-MS of deglycosylated SigmaMAb ADC Mimic heavy and light chains after reduction



50 mM DTT for 1 hour at 37 °C
2 mm × 30 cm TSKgel SuperSW3000 SEC column
isocratic 70 µL/min, 30% ACN with 0.1% TFA
2 µg injection
Xevo G2 QToF 400–4000 m/z

Due to the hydrophobicity of the dansyl-LC-SMCC attachment, the SigmaMAb ADC Mimic can also be separated into distinct drug-load peaks by HPLC hydrophobic interaction chromatography (see Figure 4).

Figure 4.
Hydrophobic Interaction Chromatography HPLC (HIC-HPLC) of SigmaMAb ADC Mimic



4.6 mm × 10 cm TSKgel butyl-NPR column run at 0.4 mL/min
A: 50 mM PO_4, pH 7.0, 1.5 M $(NH_4)_2SO_4$, 5% IPA
B: 50 mM PO_4, pH 7.0, 20% IPA
20% B for 5 min, 20%→70% B for 40 min, 70% B for 10 min
Maximum protein absorbance at 280 nm

Each vial of SigmaMAb ADC Mimic contains 500 µg of lyophilized antibody-fluorophore conjugate in phosphate-buffered saline, pH 6.7.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Reconstitute the contents of the vial by adding 100 µL of ultrapure water and mixing thoroughly, resulting in a 5 mg/mL phosphate buffered solution.

The solubilized product has been verified to be stable at 2–8 °C for one week and at –20 °C for one month. The solubilized product can be further diluted as needed at the time of use.

References
1. Wagner-Rousset, E. *et al*., Antibody-drug conjugate model fast characterization by LC-MS following IdeS proteolytic digestion. MAbs., Jan-Feb; **6(1)**, 173-84 (2014).
2. Rodriguez-Aller, M. *et al*., Practical method development for the separation of monoclonal antibodies and antibody-drug-conjugate species in hydrophobic interaction chromatography, part 1: optimization of the mobile phase. J. Pharm. Biomed. Anal., Jan 25; **118**, 393-403 (2016).
3. Wakankar, A. *et al*., MAbs., Analytical methods for physicochemical characterization of antibody drug conjugates. Mar-Apr; **3(2)**, 161-72 (2011).

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