OviGenex
PREMIUM OVINE COLLAGEN

Technical Bulletin
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CollOvine-PEG-MAL to rAspf9

Introduction

Maleimide conjugation chemistries are commonly used to generate covalent conjugates with sulfhydryl-containing molecules. The primary amine groups (lysine residues) of CollOvine are reacted with a heterobifunctional crosslinker to yield collagen derived with a maleimide group (CollOvine-PEG2-MAL), which is then reactive with other species containing free sulfhydryl groups (*e.g.*, cysteine residues). Cysteine residues commonly occur naturally in protein sequences or can easily be engineered into the protein or peptide sequence.

We have successfully conjugated CollOvine-PEG2-MAL to rAspf9, an *Aspergillus fumigatus* cell wall glucanase protein. The rAspf9 was recombinantly prepared in-house in an *E. coli* expression vector. The resulting protein is ~29 kDa, with one cysteine residue close to the N-terminus. The rAspf9 protein has been shown to be an effective antigen in a developmental *A. fumigatus* vaccine (AspaVax) by our collaborative partner, Molecular Express, Inc.

Procedure

To prepare the cysteines of the rAspf9 for conjugation, one volume of rAspf9 at ~ 2 mg/mL was reduced with 0.1 volume of 2-mercaptoethanol for ~30 minutes at room temperature. The excess 2-mercaptoethanol was removed by passage through a desalting column (*e.g.*, Illustra NAP-5 column). The peak fractions were determined by absorbance at 280 nm. The reduction of the cysteine residues was confirmed by Ellman's reagent. To avoid re-oxidation of the cysteines, the reduced rAspf9 protein was used immediately.

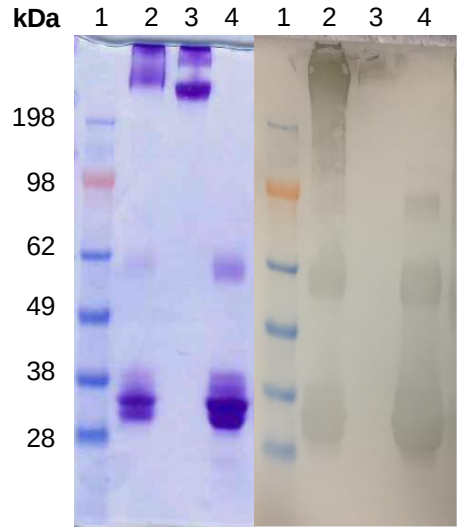

Figure 1. SDS-PAGE and Western Blot of CollOvine-rAspf9 conjugation. Lane 1: Protein Marker; Lane 2: CollOvine-PEG2-MAL and rAspf9; Lane 3: CollOvine-PEG2-MAL only; Lane 4: rAspf9 only. The image on the left is the Coomassie-stained SDS-PAGE analysis of the samples indicated. The image of the right is the Western Blot against rAspf9 of the samples indicated.

One mole of CollOvine-PEG2-MAL was mixed with about 5 mole excess of reduced rAspf9 protein. The reaction was incubated for 30 minutes at room temperature. The conjugates were analyzed as described below.

Results

The conjugate was analyzed by SDS-PAGE (**Figure 1, left**) and Western Blot (**Figure 1, right**). CollOvine-PEG2-MAL, rAspf9 and CollOvine-PEG2-MAL reacted with rAspf9 were loaded on an SDS-PAGE gel. One gel was stained with Coomassie stain to indicate the molecular weights of the samples (**Figure 1, left**). Another gel with the same samples was analyzed by Western Blot (**Figure 1, right**) following a standard procedure. The primary antibodies were anti-rAspf9 antibodies obtained from the serum of mice vaccinated with AspaVax, the secondary antibodies were goat anti-mouse IgG1 antibodies conjugated to horse radish peroxidase (ThermoFisher Scientific), and the substrate was the chromogenic CN/DAB substrate (ThermoFisher Scientific).

As shown in lane 2 of the SDS-PAGE (**Figure 1**, **left**), the CollOvine-PEG2-MAL-rAspf9 conjugate has a higher molecular weight than the CollOvine-PEG2-MAL alone (lane 3) or rAspf9 alone (lane 4). Since an excess of rAspf9 was added, unconjugated rAspf9 (~30 kDa band) remained in lane 2. The Western Blot results (**Figure 1, right**) show that the anti-rAspf9 antibodies are reactive against the rAspf9 (see lanes 2 and 4, ~30 kDa). The dark color at the higher molecular weight in lane 2 (**Figure 1, right**) confirms the presence of CollOvine-PEG2-MAL-rAspf9, and that the CollOvine-PEG2-MAL was conjugated with multiple copies of the rAspf9 protein.