OviGenex
PREMIUM OVINE COLLAGEN

Technical Bulletin
Updated on January 12, 2021

CollOvine-SPDP conjugation to rAspf9

Introduction

CollOvine-SPDP is provided as one of our ovine collagen derivatives for the covalent conjugation with sulfhydryl-containing molecules. The primary amine groups (lysine residues) of CollOvine are reacted with succinimidyl 3-(2-pyridyldithio)propionate (SPDP) to yield collagen derived with a cleavable disulfide bond (CollOvine-SPDP), the cleaved linker will then be 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-SPDP 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.

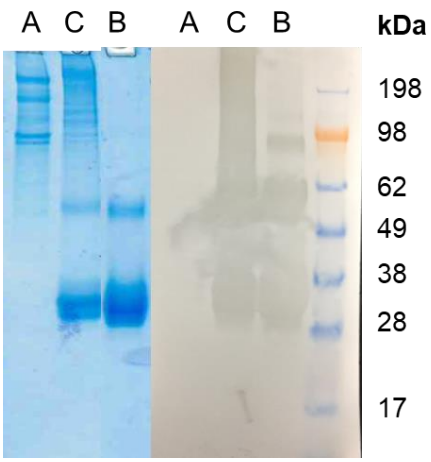

Figure 1. SDS-PAGE and Western Blot of CollOvine-SPDP conjugated to rAspf9 conjugation. Lane A: CollOvine-SPDP only; Lane B: rAspf9 only; Lane C: CollOvine-SPDP conjugated to rAspf9. The image on the left (blue hue) is the Coomassie-stained SDS-PAGE analysis of the samples indicated. The image of the right (black and white hue) is the Western Blot against rAspf9 of the samples indicated.

Prior to conjugation, the CollOvine-SPDP was reduced with 2-mercaptoethanol as well. The reduction of the CollOvine-SPDP can be confirmed by the absorbance of the resulting pyridine 2-thione molecules at 343 nm. The pyridine 2-thione molecules were removed by a desalting centrifugal filter. One mole of CollOvine-SPDP 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-SPDP, rAspf9 and CollOvine-SPDP 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 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 C of the SDS-PAGE (**Figure 1**, **left**), the CollOvine-SPDP-rAspf9 conjugate has a higher molecular weight than the CollOvine-SPDP alone (lane A) or rAspf9 alone (lane B). Since an excess of rAspf9 was added, unconjugated rAspf9 (~30 kDa band) remained in lane C. The Western Blot results (**Figure 1, right**) show that the anti-rAspf9 antibodies are reactive against the rAspf9 (see lanes C and B, ~30 kDa). The dark color at the higher molecular weights in lane C (**Figure 1, right**) confirms the presence of CollOvine-SPDP-rAspf9 thus verifying that the CollOvine-SPDP was conjugated to multiple copies of rAspf9.