

EcoCRM™ : Affordable CRM₁₉₇ Vaccine Carrier Protein

A. Lees*#, N. Oganessian*, I. Krauss**, D. Nguyen**

*Fina Biosolutions LLC, **Brandeis University, # University of Maryland

Affordable carrier protein

CRM₁₉₇, a genetically detoxified diphtheria toxin, is widely used as a carrier protein in conjugate vaccines. It was originally expressed as a secreted protein in *Corynebacterium diphtheriae*. Recently recombinant CRM₁₉₇ expressed in the periplasm of *Pseudomonas fluorescens* has become available.

Fina BioSolutions has developed a new CRM₁₉₇, EcoCRM™, produced in *E.coli*. EcoCRM™ is expressed as soluble protein in the cytoplasm of a widely-used *E.coli* expression strain. High expression of EcoCRM™ and a simple purification method allows low cost production and the promise of significantly reducing the cost of this component of conjugate vaccines.

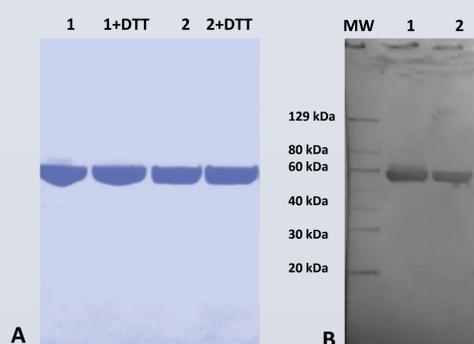
The presented data show that EcoCRM™ as a carrier protein is equivalent to CRM₁₉₇ from established sources.

Overview

- EcoCRM™ is a new affordable CRM₁₉₇ carrier protein
- Highly expressed as a soluble single polypeptide in the cytoplasm of *E.coli*
- Equivalent immunogenicity as a carrier protein to other rCRM₁₉₇
- Progress towards clinical development
 - Biophysical analysis completed by independent lab to be presented at PEGS (Boston, May 1, 2017)
 - GMP master cell banking completed
 - CMO selected
 - GMP EcoCRM™ anticipated by Q2 2018

SDS-PAGE and WB analysis of EcoCRM™ and CRM₁₉₇ (Pfenex)

EcoCRM™ is expressed as soluble properly folded single polypeptide and highly purified. Recombinant CRM₁₉₇ from Reagent Proteins (Pfenex), made in *Pseudomonas fluorescens* has generally been shown to be equivalent to “native” CRM₁₉₇ expressed by *Corynebacterium diphtheriae* (Cassidy et al., Bioprocessing Int., p.50 Nov. 2012).

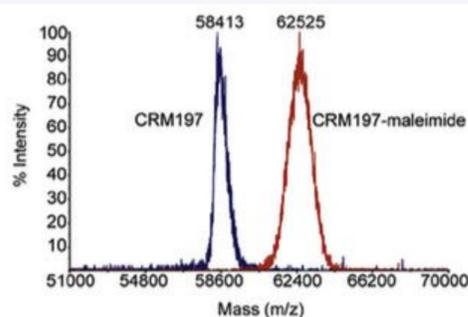


A: SDS-PAGE Reduced vs non reduced. Coomassie Blue staining. B: Western Blot analysis, detection with polyclonal rabbit anti-CRM₁₉₇ (AIC Biotech)

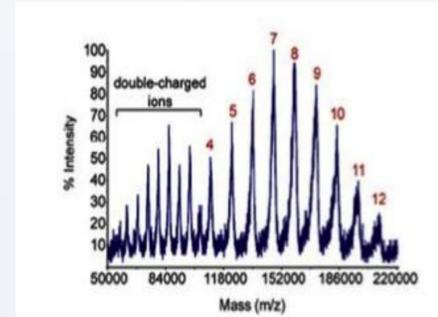
1- CRM₁₉₇ (Pfenex) 2- EcoCRM™

Glycopeptide-CRM conjugate

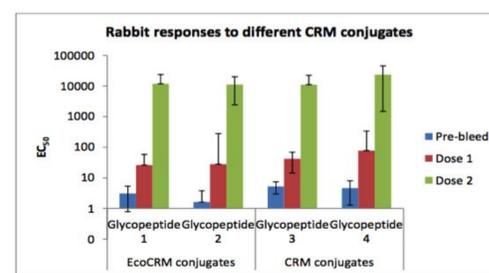
Thio-ether conjugation is frequently used for linking peptides, oligosaccharides and other antigens to a carrier protein. EcoCRM™ was derivatized with an excess of the NHS maleimide reagent sulfo-EMCS.



The MW of labeled EcoCRM™ increased from 58,413 to 62,525, a mass increase of 4,100. Each maleimide linker adds about 208 mass units, indicating about 20 of CRM's 39 lysines have been



Thiol-glycopeptide (MW ~15kDa) was conjugated to EMCS-labeled CRM. Mass spec of the conjugates indicated an average of 7 glycopeptides/CRM.



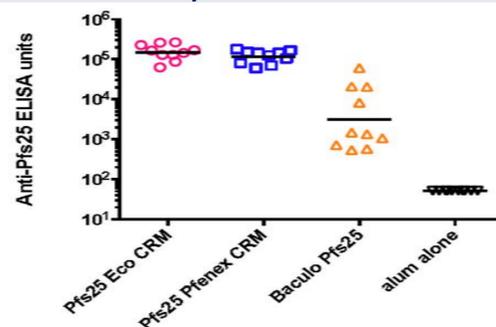
Glycopeptide conjugates of EcoCRM™ and Pfenex CRM were prepared using EMCS-labeled CRM. Rabbits were immunized twice. After the final bleed, IgG anti-glycopeptide titers were measured using glycopeptide-BSA as the ELISA antigen.

Glycopeptides conjugates with EcoCRM™ and rCRM₁₉₇ (Pfenex) induce comparable anti-glycopeptide titers.

Protein-CRM conjugate

Antibodies to the malaria protein Pfs25 have been shown to prevent the malaria parasite from passing through the mosquito gut. Poorly immunogenic by itself, Pfs25-protein conjugates induce anti-Pfs25 antibodies in mice that can block malaria mosquito transmission.

Anti-Pfs25 antibody titer



Mice were immunized 3 times with pfs25-CRM conjugates made with EcoCRM™ or Pfenex CRM. Each dose contained equal amounts of 4 µg Pfs25 in soluble or conjugate form, adsorbed to Alum.

The authors thank the Lab. Of Malaria & Vector Research at NIAID/NIH for the ELISA and SMFA and the MVI for providing Pfs25 expressed in baculovirus (Malar J 15:405, 2016).

Standard Membrane Feeding Assay

Sample name (4ug Pfs25)	IgG conc [ug/ml]	% inhibition			p-value
		estimate	95%CI Lo	95%CI Hi	
4B7	94	90.6	78.7	95.9	0.001
Gr 1: Pfs25 Eco CRM	750	100.0	99.8	100.0	0.001
Gr 2: Pfs25 Pfenex CRM	750	100.0	99.6	100.0	0.001
Gr 3: Baculo Pfs25	750	71.7	37.0	87.9	0.002
Gr 4: alum alone	750				

The ability of the antibody to block *Plasmodium falciparum* malaria parasite transmission in the mosquito gut was evaluated using the “Standard Membrane Feeding Assay”, SMFA; (Miura et al. Vaccine 34:4145, 2016). Antibody 4B7 is a positive control.

Conjugates with EcoCRM™ Pfenex CRM induced comparable anti-Pfs25 antibody response in mice and were equally effective at killing malaria parasites in a membrane feeding assay (SMFA).

Acknowledgments: Pfs25 conjugation was funded by the PATH Malaria Vaccine Initiative. The pfs25 assays were performed by the SMFA reference center at the NIAID/NIH.

For more information: www.FinaBio.com