CRM_{197} is superior to KLH as a carrier protein

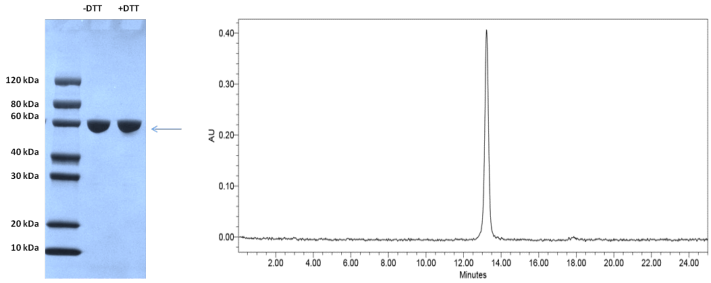
Improved Immune Response and Efficacy
with Fina Biosolutions' CRM_{197}, EcoCRM®.

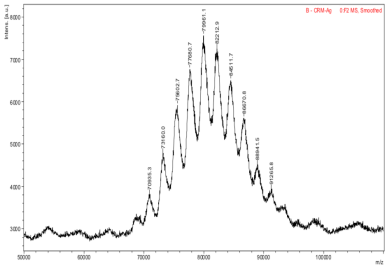
KLH is often used as a carrier protein because of its extreme immunogenicity and easy availability. However, due to its mullosk origin and poor characterization, native KLH is not an ideal carrier. In contrast, CRM_{197}, genetically detoxified diphtheria toxin, is a recombinant protein of 58.4 kDa and is also highly immunogenic. Drug and peptide CRM_{197}-conjugates can easily be characterized by MALDI-TOF mass spec. Here we show that the immunogenicity of conjugates made with Fina Biosolutions' CRM_{197}, EcoCRM®, are equal or superior to conjugates of KLH.

Keyhole limpet hemocyanin (KLH) is often used as a carrier protein in experimental vaccines. As an extremely large and foreign protein, KLH induces very high antibody titer both to itself and to molecules linked to it. It is inexpensive and the GMP material is available for clinical use. However, KLH is obtained from the blood of sea snails, making scale-up challenging. It is a messy, somewhat ill-defined polymeric protein, which makes it difficult to characterize. Furthermore, KLH solubility is poor in low salt and neutral pH solutions and quality control of KLH conjugates is problematic. There are currently no licensed vaccines that use KLH as the carrier protein. In contrast, CRM_{197}, a genetically detoxified diphtheria toxin, is produced recombinantly in bacteria as a well-defined protein of 58.4 kDa. CRM_{197} is used in multiple licensed vaccines, including Prevnar®. Fina Biosolutions has developed a unique *E. coli* strain that expresses CRM_{197} as a soluble, properly-folded, intracellular protein at high yield. FinaBio has appropriately priced their CRM_{197} for both research and clinical use. The protein is marketed under the trade name "EcoCRM®" for *E. coli* CRM_{197}.

We have consistently found that EcoCRM® conjugates induced antibody responses that were equal to or greater than KLH conjugates. Furthermore, unlike KLH, CRM_{197} conjugates can be characterized by SDS PAGE and Mass Spec.

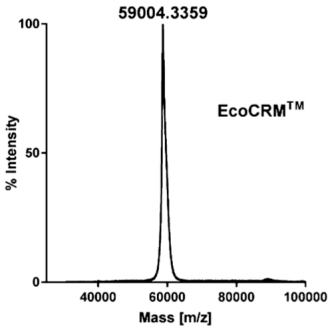
Characterization of EcoCRM® and conjugates.



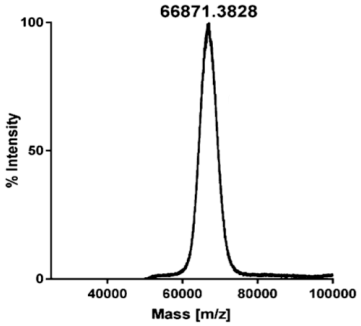


A. SDS PAGE EcoCRM® B. SEC HPLC C. MALDI-TOF of Peptide-CRM conjugate

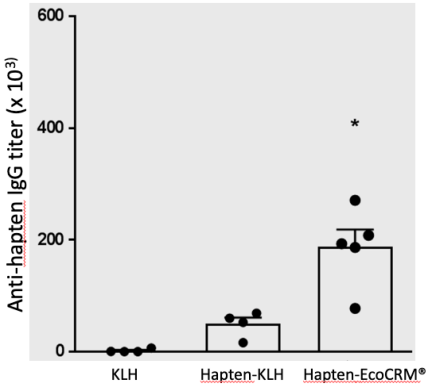
Example 1. Hapten conjugates



A1, EcoCRM®



A2. Hapten-EcoCRM®



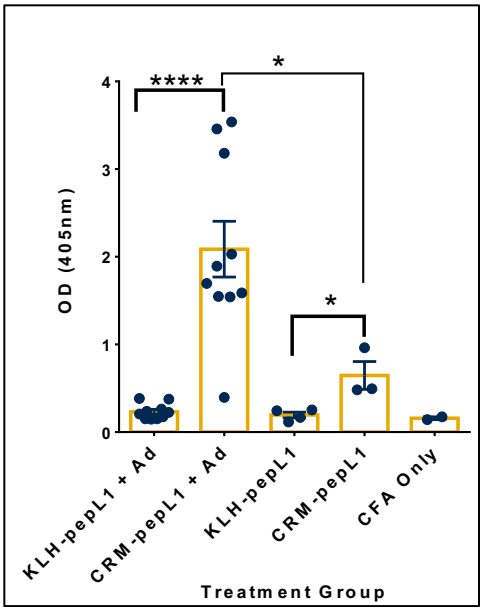
B. Anti-Hapten IgG titer

A. Characterization of carrier protein and conjugates by MALDI-TOF. EcoCRM® carrier protein was analyzed (A1) before and (A2) after conjugation to the hapten. Samples were analyzed using an Applied Biosystems/MDS SCIEX 54800 MALDI TOF/TOF analyzer operated in high-mass linear mode.
B. Antibody Hapten IgG responses. BALB/c mice (n ≥ 4/group) were immunized s.c. with 100 µg unconjugated KLH (control), Hapten-KLH, or Hapten-EcoCRM®, formulated with 1 mg of alum adjuvant on days 0, 14 and 28. Titers we determined by ELISA.

Data courtesy of Dr. Marco Pravetoni, U Minn

Example 2. Peptide Conjugates

Peptide PepL1 was conjugated to FinaBio's Ready-to-Conjugate™ CRM-maleimide or to KLH (by the peptide synthesis company). Ready-to-Conjugate™ CRM-maleimide greatly facilitated synthesis of the EcoCRM® conjugate. As the figure shows, the CRM conjugate gave an excellent anti-peptide antibody response compared with the KLH conjugate.



Immune response in mice developed after vaccination and boost with a peptide-protein+/-adjuvant that targets an OMP on the surface of *Burkholderia*. AddaVax adjuvant (~MF59). The response to conjugate made with FinaBio's Ready-to-Conjugate™ CRM was significantly higher than to the KLH. Note also that the CRM conjugate was immunogenic even in the absence of adjuvant.

Data courtesy of Dr. Slawomir Lukomski, West Virginia University

For more information please visit our website, FinaBio.com, or email Info@FinaBio.com