



EcoCRM™ -Economical Recombinant CRM₁₉₇

Significantly reducing the cost of conjugate vaccines

Natalia Oganessian* , Sanjay Jain**, Andrew Lees*

*Fina Biosolutions LLC, Rockville, MD, USA, ** Novo Biosolutions Inc., Shrewsbury, MA, USA

AFFORDABLE CARRIER PROTEIN

CRM₁₉₇, a genetically detoxified diphtheria toxin, is widely used as a carrier protein in conjugate vaccines. Highly effective conjugate vaccines against *Streptococcus pneumoniae*, *Haemophilus influenzae* b and *Neisseria meningitidis* have been made using this carrier protein. CRM₁₉₇ has been expressed as a secreted protein in *Corynebacterium diphtheriae* but effective CRM₁₉₇ production requires precise control of growth parameters and yields are typically low (<100 mg/L). Recently, CRM₁₉₇ expressed in the periplasm of *Pseudomonas fluorescens* has become available, but it is not generally priced to be an affordable alternative.

Fina BioSolutions has developed a highly efficient *Escherichia coli* expression system for CRM₁₉₇, along with a simple purification scheme. Previous methods for CRM₁₉₇ expression in *E.coli* have involved refolding from insoluble inclusion bodies or secretion into the periplasm. We have developed a method to produce soluble CRM₁₉₇ in the cytoplasm of *E.coli* and achieved expression yield of grams per liter in a fermenter. The production process comprises of a simple highly efficient purification method with minimal number of steps. EcoCRM™, economical CRM from *E. coli*, offers the promise of significantly reducing the cost of conjugate vaccines.

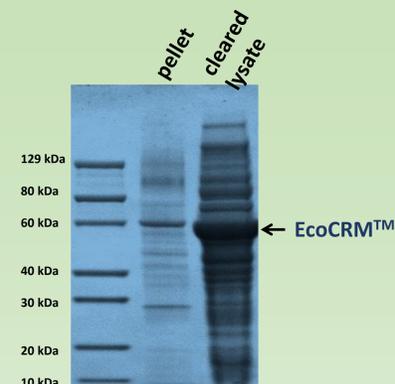
SUMMARY

EcoCRM™ is a new and affordable CRM₁₉₇

- ◆ Simple, low cost fermentation in well-accepted *E. coli* strain
- ◆ Straightforward purification process
- ◆ High yields of soluble CRM₁₉₇
- ◆ Native sequence
- ◆ No nicking
- ◆ Excellent solubility properties

EXPRESSION

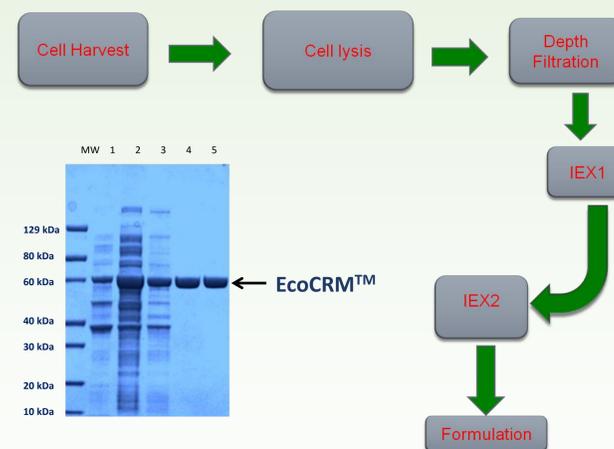
EcoCRM™ is expressed in *E. coli* intracellularly as a soluble protein. High expression yield of correctly folded* CRM₁₉₇ is achieved by using an *E. coli* strain developed by Fina BioSolutions, along with an optimized coding sequence.



* Fina BioSolutions has developed an ELISA method to evaluate CRM₁₉₇ functional integrity.

PURIFICATION

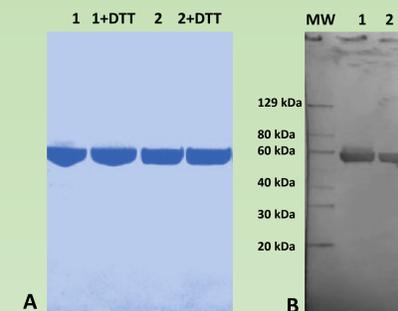
EcoCRM™, expressed in *E.coli*, is purified using a straightforward and economical purification scheme to more than 95% purity



1-pellet; 2-cleared lysate; 3-CRM₁₉₇ after IEX1, 4-EcoCRM™ after IEX2, 5µg, 5-EcoCRM™ after IEX2 +DTT

SDS-PAGE and Western Blot

EcoCRM™ is expressed in *E.coli* as an intact single chain polypeptide.

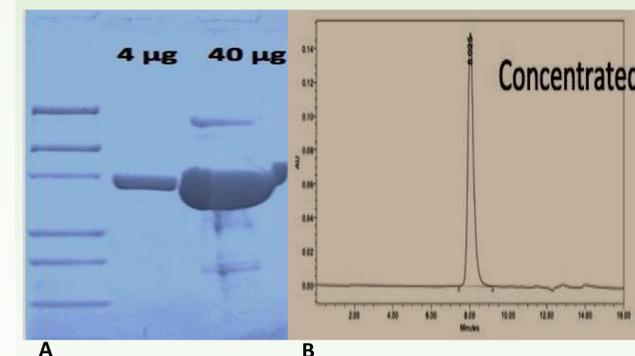


1- CRM₁₉₇ (*Pseudomonas*) 2- EcoCRM™ (*E. coli*)

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

SOLUBILITY

EcoCRM™ can be concentrated to >30 mg/ml, in PBS, without precipitation or oligomerization.



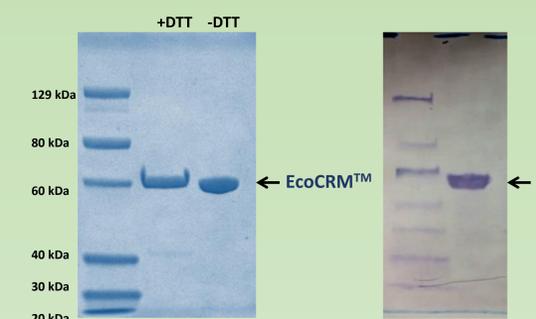
A: SDS-PAGE analysis of concentrated EcoCRM™ B: SEC-HPLC analysis of concentrated EcoCRM™

MASS SPEC ANALYSIS

CRM₁₉₇ native sequence: 58,412 Da
EcoCRM™: 58,413 Da

STABILITY

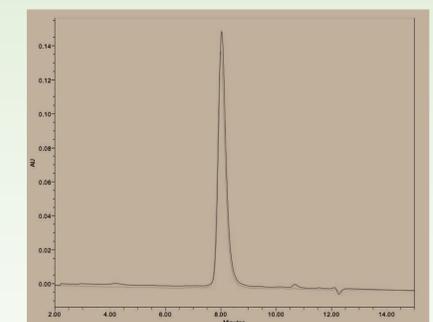
EcoCRM™ shows minimal "nicking" after storage at 4°C for 60 days and no sign of degradation.



A: SDS-PAGE analysis B:Western Blot analysis, detection with polyclonal rabbit@CRM₁₉₇ (AIC Biotech) Additional stability studies are underway.

LONG TERM STORAGE

EcoCRM™ can be stored at -80°C with a cryoprotectant. The thawed protein remains monomeric.



SEC-HPLC analysis: overlay of EcoCRM™ samples before and after freezing. Lyophilization studies are underway.

ACKNOWLEDGMENTS

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CONTACT: Fina BioSolutions LLC
Andrew Lees alees@finabio.com
301 633-0464 www.FinaBio.com