



# EcoCRM™ -Economical Recombinant CRM<sub>197</sub>

## Significantly reducing the cost of conjugate vaccines

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### AFFORDABLE CARRIER PROTEIN

CRM<sub>197</sub> , 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. CRM<sub>197</sub> has been expressed as a secreted protein in *Corynebacterium diphtheriae* but effective CRM<sub>197</sub> production requires precise control of growth parameters and yields are typically low (<100 mg/L). Recently, CRM<sub>197</sub> 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 CRM<sub>197</sub>, along with a simple purification scheme. Previous methods for CRM<sub>197</sub> expression in *E.coli* have involved refolding from insoluble inclusion bodies or secretion into the periplasm. We have developed a method to produce soluble CRM<sub>197</sub> 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.

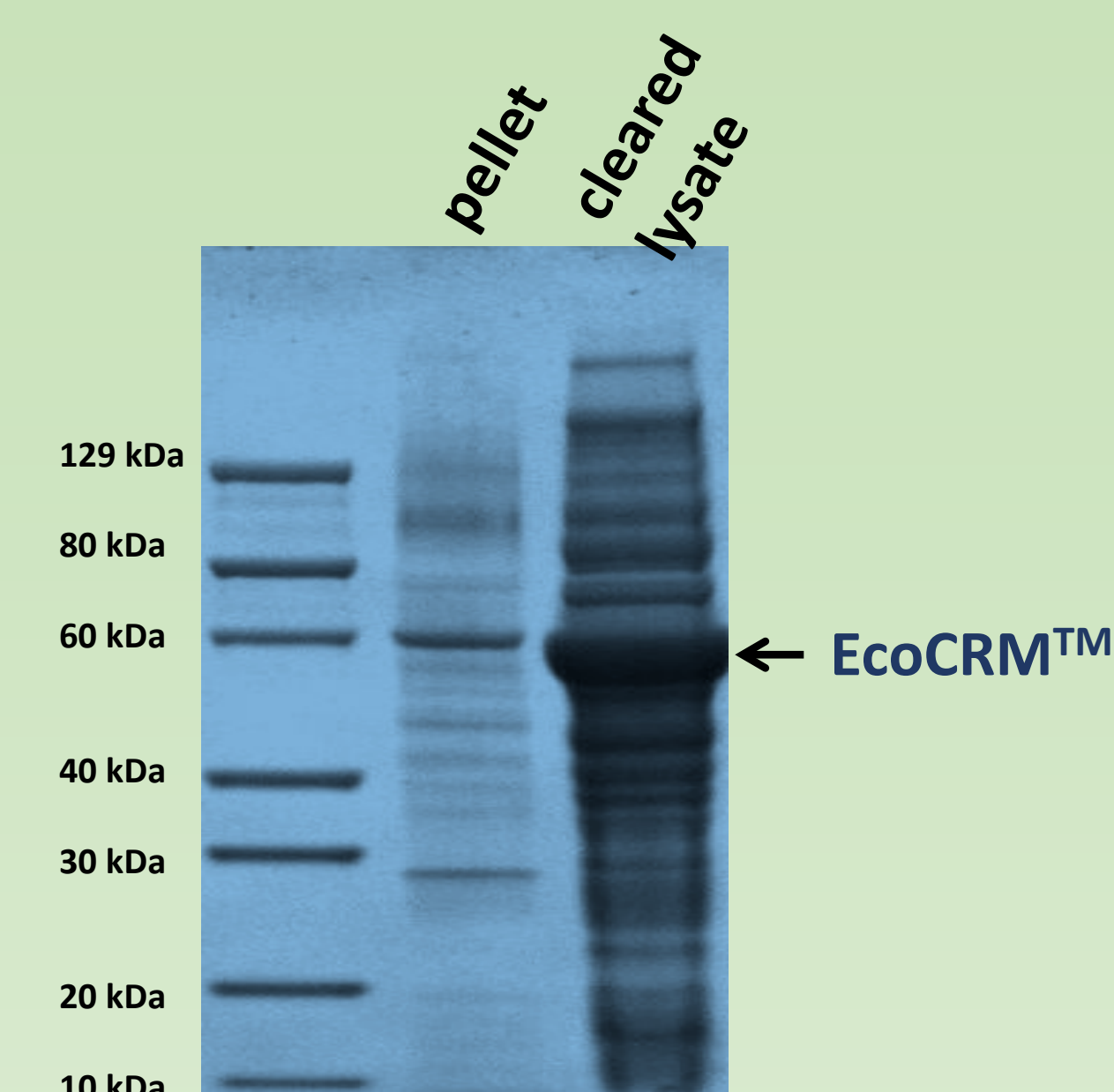
### SUMMARY

EcoCRM™ is a new and affordable CRM<sub>197</sub>

- ◆ Simple, low cost fermentation in well-accepted *E. coli* strain
- ◆ Straightforward purification process
- ◆ High yields of soluble CRM<sub>197</sub>
- ◆ Native sequence
- ◆ No nicking
- ◆ Excellent solubility properties

### EXPRESSION

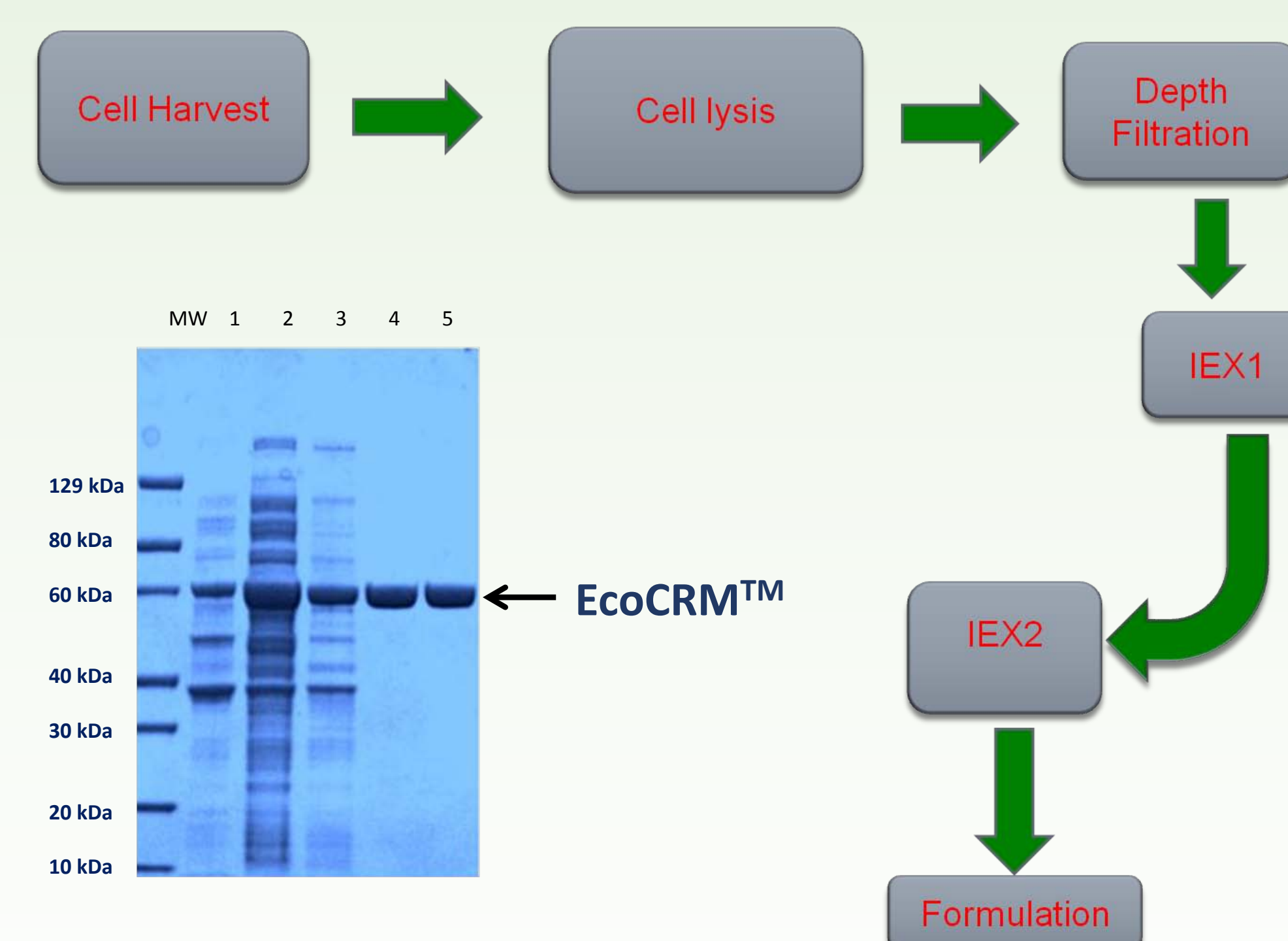
EcoCRM™ is expressed in *E. coli* intracellularly as a soluble protein. High expression yield of correctly folded\* CRM<sub>197</sub> is achieved by using an *E. coli* strain developed by Fina BioSolutions, along with an optimized coding sequence.



\* Fina BioSolutions has developed an ELISA method to evaluate CRM<sub>197</sub> functional integrity.

### PURIFICATION

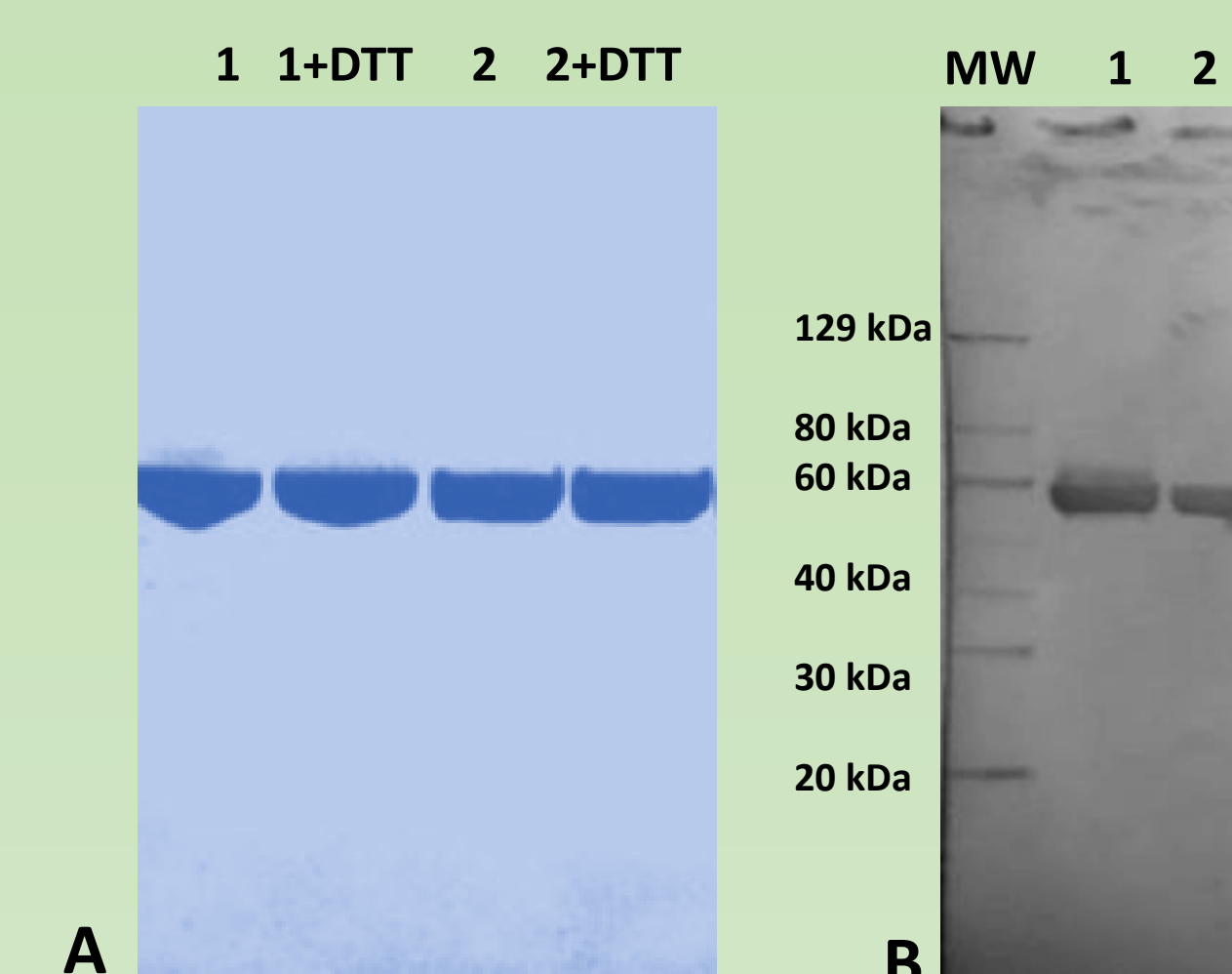
EcoCRM™, expressed in *E.coli*, is purified using a straightforward and economical purification scheme to more than 95% purity



1-pellet; 2-cleared lysate; 3-CRM<sub>197</sub> after IEX1, 4-EcoCRM™ after IEX2, 5-µg, 5-EcoCRM™ after IEX2 +DTT

### SDS-PAGE and Western Blot

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

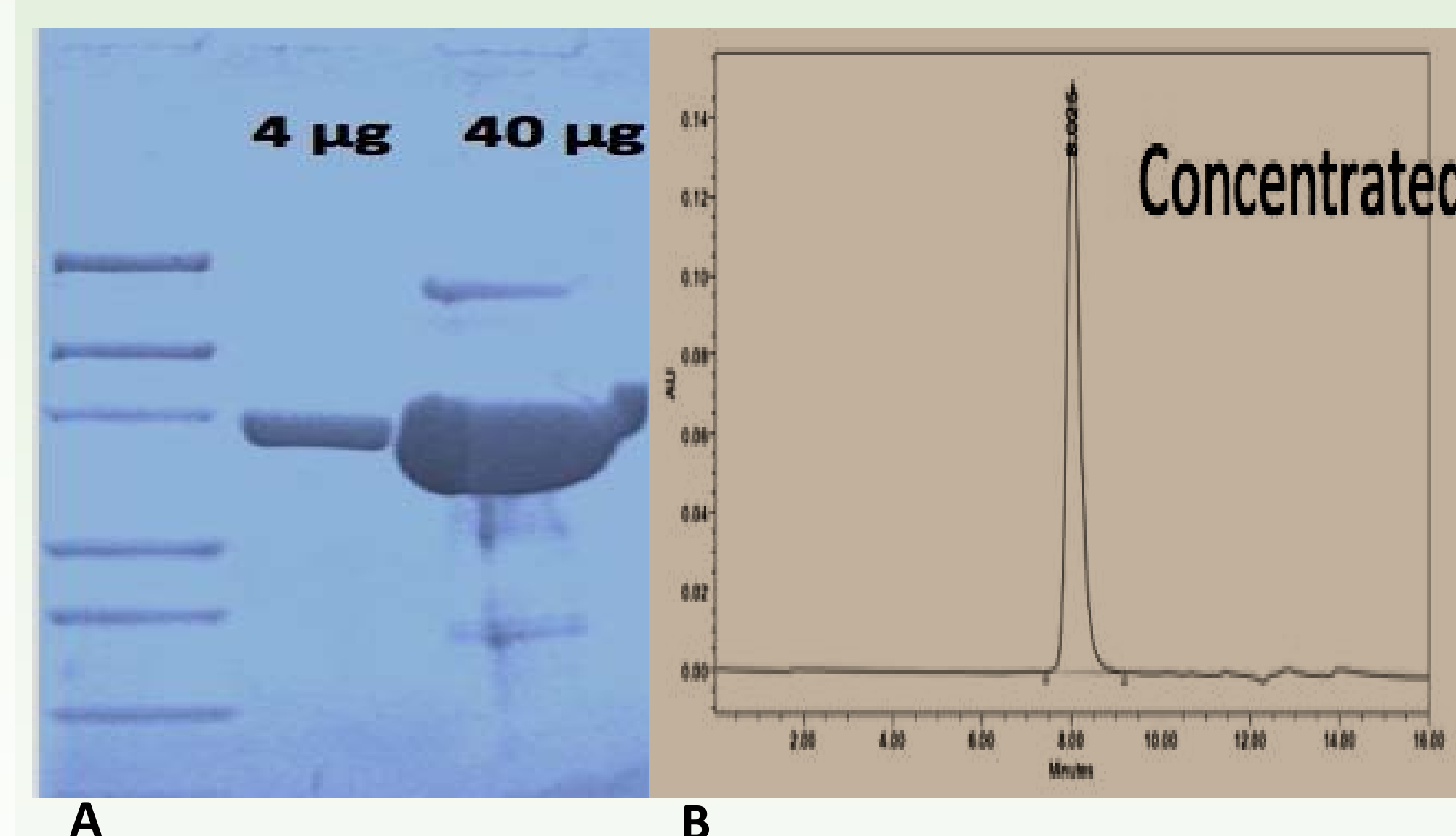


1- CRM<sub>197</sub> (*Pseudomonas*) 2- EcoCRM™ (*E. coli*)

A: SDS-PAGE Reduced vs non reduced. Coomassie Blue staining.  
B: Western Blot analysis, detection with polyclonal rabbit@CRM<sub>197</sub> (AIC Biotech)

### SOLUBILITY

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



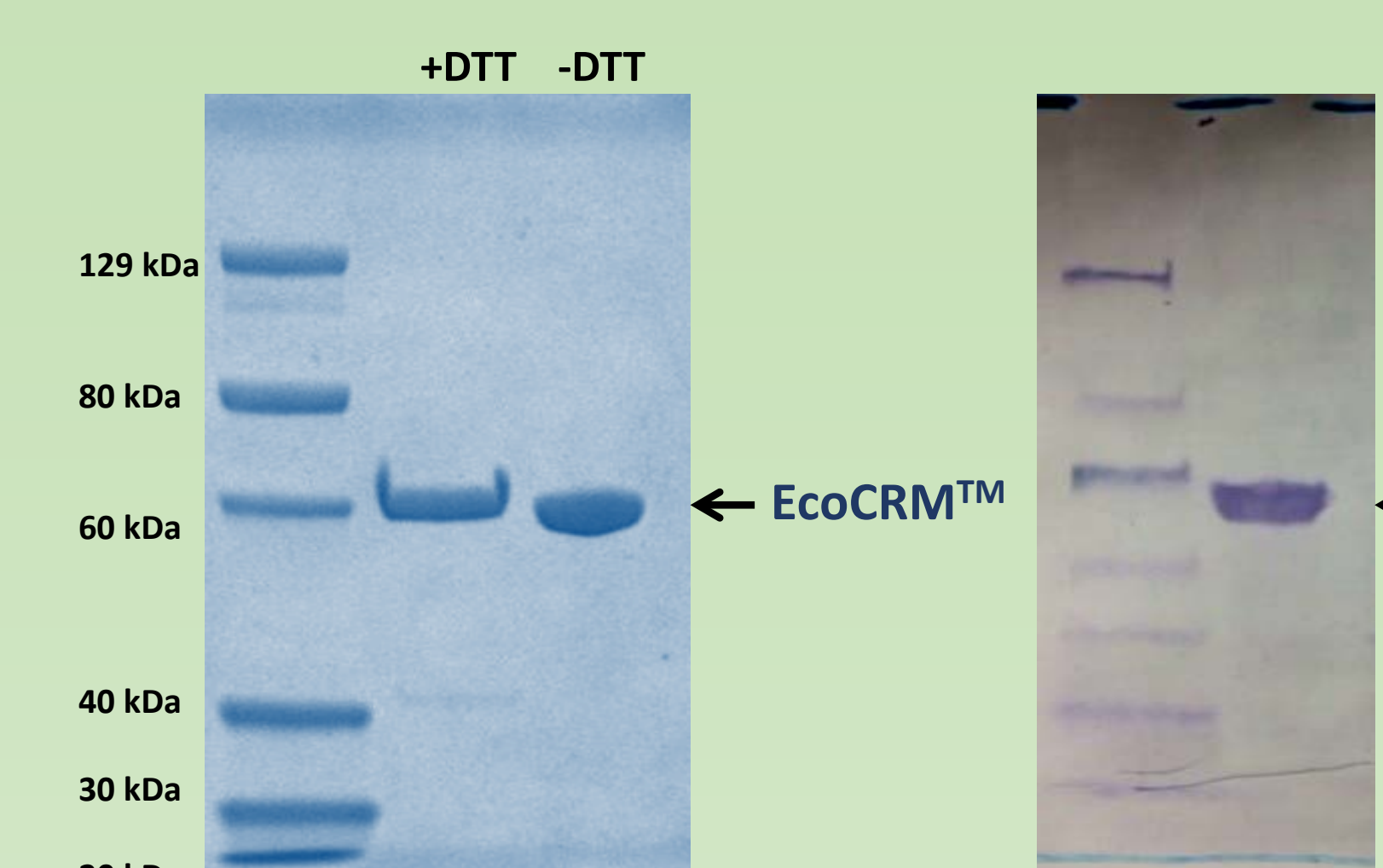
A: SDS-PAGE analysis of concentrated EcoCRM™  
B: SEC-HPLC analysis of concentrated EcoCRM™

### MASS SPEC ANALYSIS

CRM<sub>197</sub> native sequence: 58,412 Da  
EcoCRM™: 58,413 Da

### STABILITY

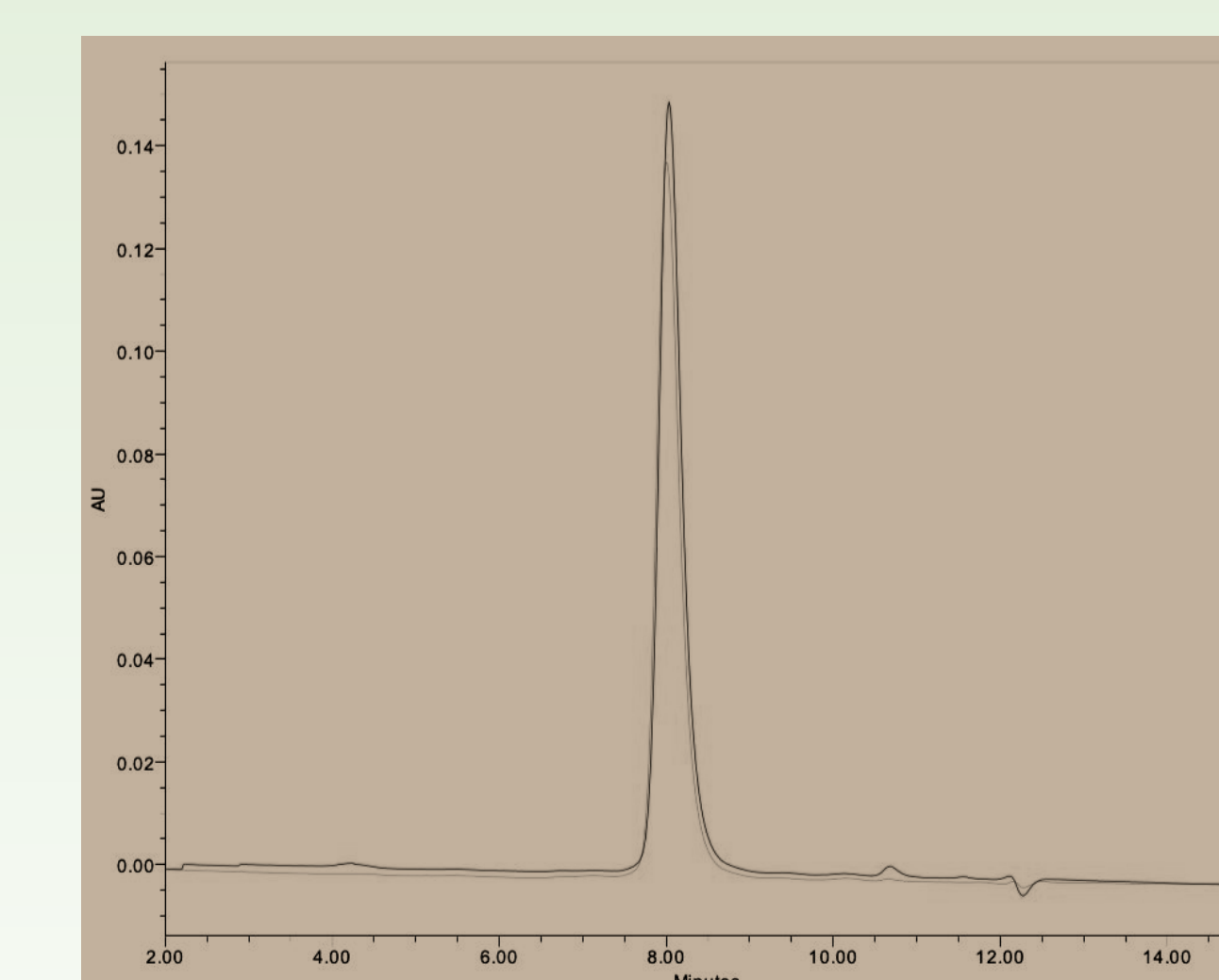
EcoCRM™ shows minimal “nicking” after storage at 4°C for 60 days and no sign of degradation.



A: SDS-PAGE analysis  
B: Western Blot analysis, detection with polyclonal rabbit@CRM<sub>197</sub> (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

**Frank Robb (U Maryland)** for contributions to the DNA work and helpful discussions.  
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