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EcoCRM197® and 8MTT, expressed in an engineered E. coli strain
and use as conjugate vaccine carrier proteins**

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GENETICALLY DETOXIFIED DIPHTHERIA AND TETANUS TOXINS (CRM₁₉₇ and 8MTT) EXPRESSED IN AN ENGINEERED *E. COLI* AND THEIR USE AS CONJUGATE VACCINE CARRIER PROTEINS

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We have developed a novel *E. coli* expression system, which we call the Gor/Met strain. Gor/Met *E. coli* has an oxidative cytoplasm and can express disulfide-bonded proteins as intracellular, soluble, properly-folded proteins. In addition to the oxidative cytoplasm, the strain has been engineered to efficiently cleave the N-terminal methionine found on cytoplasmic *E. coli* proteins. To create an oxidative cytoplasm, we deleted the gor gene in BL21 *E. coli*, resulting in an oxidative cytoplasm. We used the gor- strain to produce the conjugate vaccine carrier protein CRM₁₉₇ in a fermenter. CRM₁₉₇ was expressed as a soluble, properly folded protein in the cytoplasm and no inclusion bodies were formed. We developed a simple purification process that resulted in 2 g of purified protein per liter fermentation broth. FinaBio's CRM₁₉₇ (marketed as EcoCRM®) has been extensively compared to CRM₁₉₇ obtained from multiple manufacturers (Hickey et al., J Pharm Sci. 107, 1806, 2018). It was found to have the correct disulfide bonding. EcoCRM® is being used for several conjugate vaccines in development targeting *S. pneumoniae*, Group B Strep, malaria and vaccines for drugs of abuse. Clinical grade EcoCRM® is available now. To further improve the strain, the deleted gor gene was replaced with a methionine peptidase with the same tac promoter as the plasmid containing the recombinant protein gene. Induction induced co-expression of the methionine amino peptidase along with the heterologous recombinant protein on the plasmid. Gor/Met *E. coli* achieves can grow to high cell densities in a fermenter, unlike comparable strains, making it commercially viable for recombinant protein expression. Remarkably, Gor/Met can grow to >300g/L in fed-batch fermentation. We have initially used the Gor/Met strain to produce a unique, genetically detoxified tetanus toxin (8MTT, Przedpelski et al. mBio Aug 11;11(4):e01668-20). 8MTT is purified at >0.5 g/L fermentation and was found by mass spec analysis to have extremely low levels of N-terminal methionine, showing that the amino acid was efficiently cleaved by the peptidase. 8MTT was found to be comparable to tetanus toxoid as a carrier protein. Gor/Met *E. coli* was used to express CRM₁₉₇, tetanus toxin heavy chain fragment C and human IL-10. This work shows the potential of the Gor/Met *E. coli* to be a commercially viable production strain for producing high yields of disulfide-bonded proteins, with their native sequence. Among these are carrier proteins such as CRM₁₉₇ and a new genetically detoxified tetanus toxin, along with many therapeutic proteins of interest. The availability of affordable conjugate vaccine carrier proteins produced in a low-cost expression system makes the development of conjugate vaccines more feasible. The variety of applications including vaccines for malaria, S. pneumonia, Covid and other infectious diseases as well as vaccines for areas as diverse as drugs of abuse, cancer and cholesterol control.