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Fina Biosolutions LLC

WHITE PAPER Stability Study Summary:

Capillary-Assisted Vitrification of CRM197

Introduction

Cross-reacting material 197 (CRM197) is a nontoxic mutant of diphtheria toxin widely used as a carrier protein in polysaccharide-protein conjugate vaccines. As documented in the literature, CRM197 supports robust T-cell–dependent responses, enabling improved immunogenicity of otherwise poorly immunogenic bacterial polysaccharides. CRM197 contains two disulfide-linked domains and maintains a primarily alpha-helical structure that supports its function in conjugate vaccines.

Despite its utility, CRM197 is known to undergo several degradation pathways during manufacturing and storage. These include proteolytic clipping ("nicking") of the A and B fragments, thermal or chemical denaturation, and self-association or aggregation. Such degradation pathways can impair conjugation efficiency, reduce immunogenicity, or increase the risk of adverse events in final vaccine formulations.

Capillary-assisted vitrification (CAV) is a novel stabilization technique that rapidly transitions biomolecules from a liquid state to a highly stable, amorphous glass. Once dried, proteins and other biomolecules are typically stable for months or years at temperatures of 50°C or higher.

Working with our partners at Fina Biosolution, we evaluated the stability of their CRM197 (EcoCRM®), dried via CAV and stored for six months at both room temperature and 37°C. Test results obtained on CAV-stabilized CRM197 were compared to CRM197 stored under the standard storage condition (frozen liquid). No evidence of degradation was detected. Additionally, CAV-stabilized CRM-197 was deliberately exposed to temperatures above the reported melting temperature or T_m of the protein (41–44°C depending on pH) and the impact on the protein structure evaluated by intrinsic tryptophan fluorescence showed there was no evidence of structural change.

Methods

CRM197 was vitrified transitioning the protein from a liquid state to a stable glassy state. Samples were stored protected from moisture at room temperature (~22°C) or 37°C for six months. Stability was assessed at the end of the storage period using:

- **SDS-PAGE** to detect fragmentation or clipping.
- **Size exclusion chromatography (SEC)** to evaluate monomer content, aggregation, and fragments.
- **Tryptophan (Trp) fluorescence spectroscopy** to assess tertiary structure integrity and detect denaturation.

Results

SDS-PAGE Analysis

SDS-PAGE under reducing and non-reducing conditions showed **no detectable clipping** of CRM197 immediately post stabilization and again after six months of storage at both temperatures. All samples exhibited a dominant band at the expected molecular weight, comparable to the frozen control.

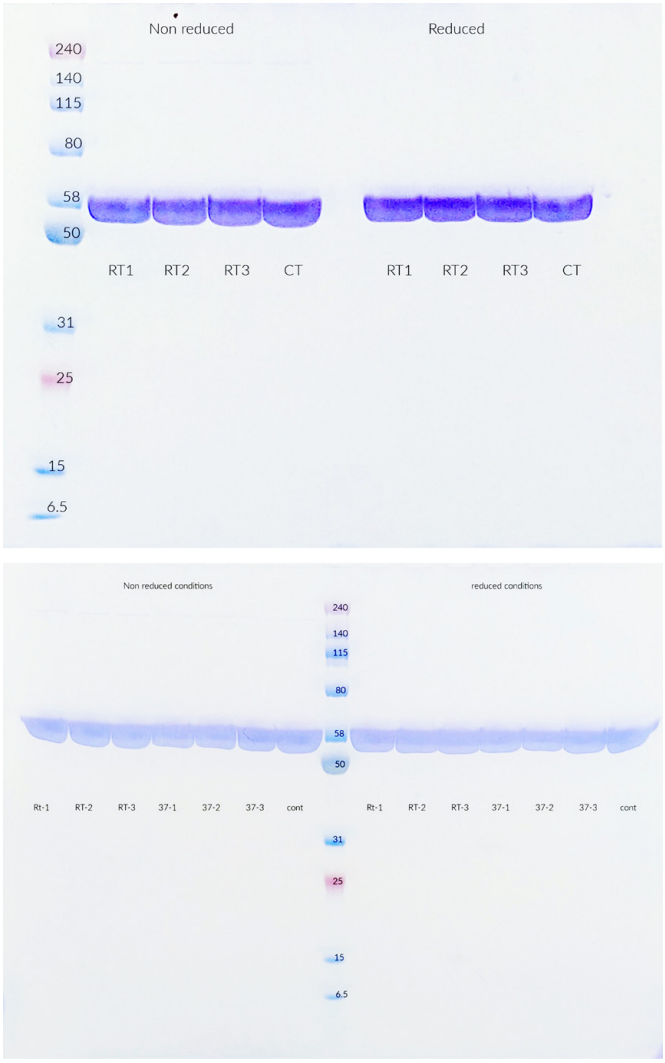

Fig 1. Evaluation of CRM197 protein integrity via SDS-PAGE. Samples were analyzed under reducing and non-reducing conditions immediately post-stabilization (top) and after six months of storage (bottom). The consistent migration pattern across all lanes indicates that the CAV-stabilized CRM197 maintains its structural identity, matching the profile of the frozen benchmark without detectable clipping or degradation.

SEC Analysis

SEC demonstrated that CRM197 remained predominantly monomeric following storage. Post stabilization, no increases in aggregate content or appearance of lower-molecular-weight fragments were observed. Profiles from both storage temperatures were essentially indistinguishable from the frozen control indicative of both excellent stability and high levels of protein recovery. Similar results were obtained at t=6M, showing no increase in either fragmentation or aggregation.

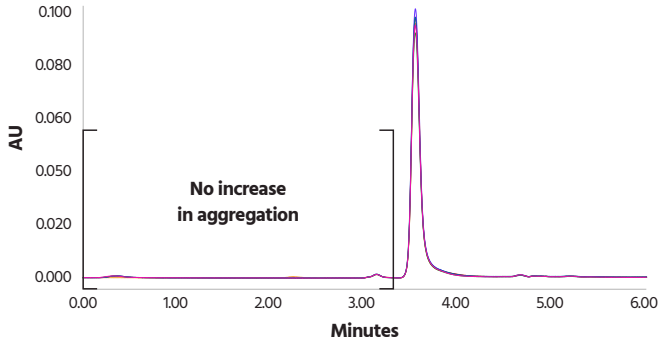

Fig 2. HPSEC results for CAV-stabilized CRM 197 stored for 6 months at 37°C. The pink, blue, and green traces represent results obtained from independent samples and show high, consistent protein recovery and monomeric size distribution.

Tryptophan Fluorescence

Intrinsic Tryptophan fluorescence can be used to monitor change in the tertiary or quaternary structure of proteins with the emission maxima typically shifting to longer wavelengths or the emission intensity decreasing as proteins unfold. When stored in a liquid format and exposed to temperatures above the T_m, CRM197 exhibits a time dependent decrease in fluorescent signal. The CAV-stabilized CRM-197 is stable and maintains consistent emission intensity.

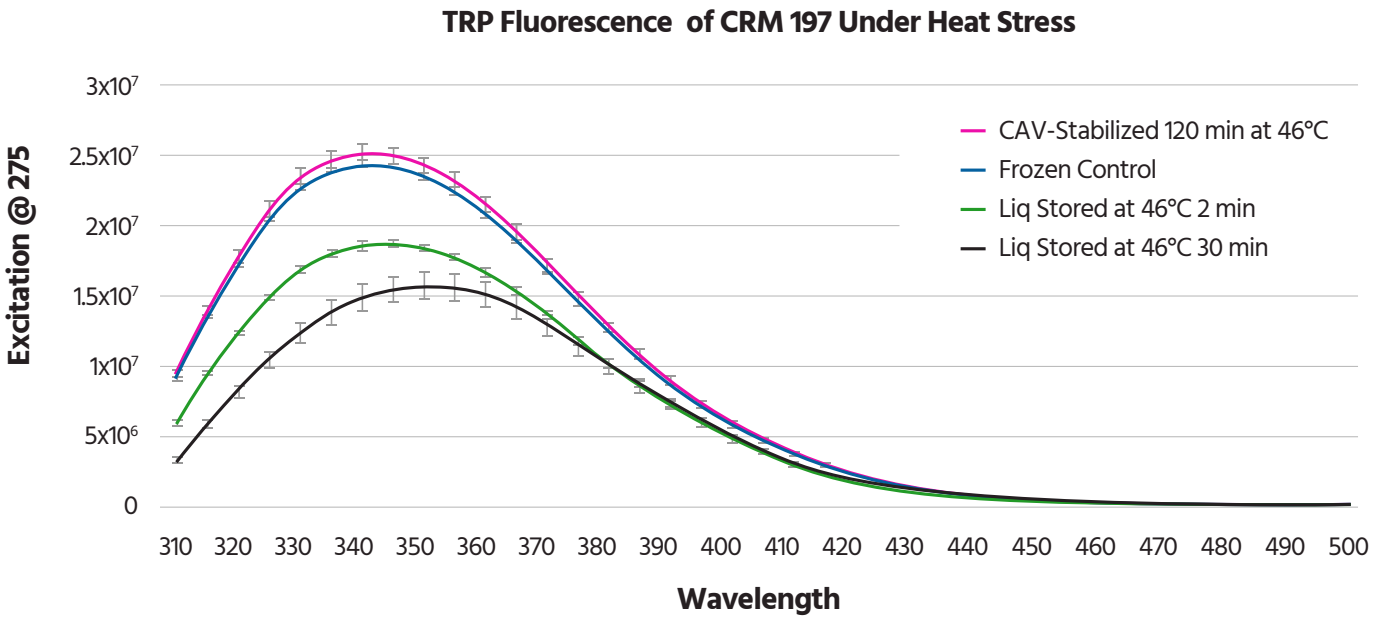

Fig 3. Relative structural integrity of CRM197. Fluorescence data highlights the superior stability of heat-stressed CAV samples compared to liquid formats. The CAV-stabilized signal tracks closely with the frozen benchmark, confirming the maintenance of the protein's native conformation.

Conclusions

Capillary-assisted vitrification produced highly stable CRM197 suitable for storage at both ambient and elevated temperatures. Across all analytical measurements—SDS-PAGE, SEC, and Trp fluorescence—there was **no evidence of clipping, denaturation, or aggregation** after six months at either room temperature or 37°C.

Given CRM197's critical role in polysaccharide conjugate vaccine manufacture and its known susceptibility to fragmentation and structural degradation, the ability to store CRM197 without refrigeration represents a significant advancement. CAV-stabilized CRM197 may reduce cold-chain constraints and improve the practicality of conjugate vaccine production.

References: Hickey JM, Toprani VM, Kaur K, Mishra RPN, Goel A, Oganesyan N, Lees A, Sitrin R, Joshi SB, Volkin DB. Analytical Comparability Assessments of 5 Recombinant CRM197 Proteins From Different Manufacturers and Expression Systems. J Pharm Sci. 2018 Jul;107(7):1806-1819. doi: 10.1016/j.xphs.2018.03.002. Epub 2018 Mar 8. PMID: 29526446.

To learn more about the benefits of CAV for your assays, visit ambientbio.com.

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