Enhancing Lipofectamine Transfection Efficiency using Capillary-Mediated Vitrification Process

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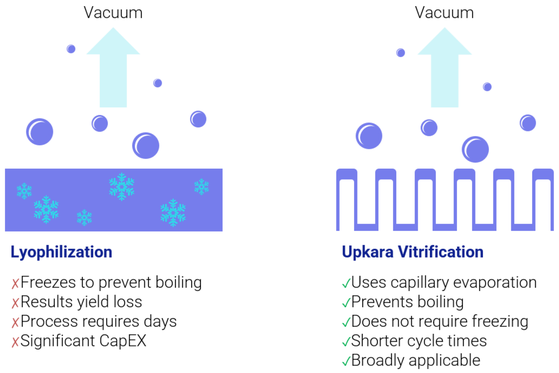
ABSTRACT

Transfecting primary mammalian cells presents challenges in achieving effective nucleic acid introduction. Lipofectamine, a widely used transfection reagent, employs a reputable lipid-based delivery method. However, difficulties persist, particularly in achieving effective mRNA transfection in challenging cell lines. Addressing this issue, we developed a technology aimed at preserving biomolecules, including mRNA, against ambient stressors without compromising its structural and functional integrity. Focusing on Lipofectamine MessengerMax as a cargo delivery system, we utilized mRNA encoding eGFP to assess transfection efficiency in Chinese Hamster Ovary (CHO) cells. Employing our novel vitrification technology and Biofix™ Buffer, we aimed to stabilize these transfection reagents on a porous scaffold for long-term storage and downstream application in cells. Our results indicate that both lipofectamine and mRNA are highly compatible with our vitrification process and buffer. Additionally, the use of stabilized lipofectamine reagent led to a greater than two-fold increase in transfection relative to the standard transfection reagents. Preliminary data also demonstrates consistently enhanced transgene expression even after storing lipofectamine at room temperature and 37°C for a month on our scaffold. These results showcase the potential of our innovative approach to enhance transfection while maintaining the functionality of the reagents.

CMV PROCESS and USE

Capillary-mediated vitrification (CMV) is a novel method that provides significant biomolecule stabilization, while being less resource intensive than lyophilization



The CMV process leverages the naturally-occurring process of capillary evaporation to rapidly remove moisture from an aqueous matrix without freezing or boiling, transitioning biological reagents into a stable, glassy state. The pores within the scaffold act as capillaries, increasing the surface area and surface tension. The increase in surface tension prevents boiling, allowing the material to be dried under vacuum without a freezing step (1-3).

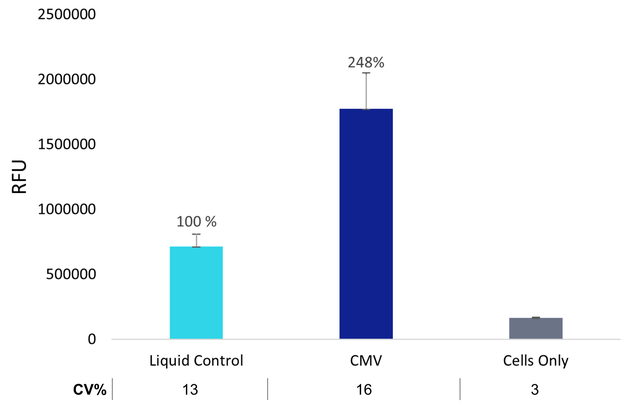
CMV Reagent Preparation

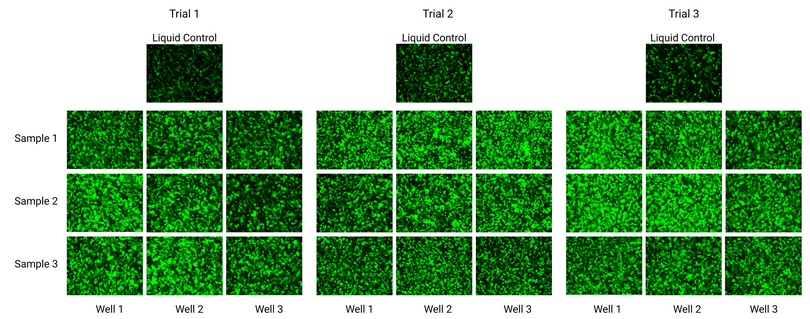
The Upkara process differs from lyophilization in that it requires minimal optimization, no freezing step, and can be completed at the bench in less than one hour. The approach is broadly applicable to variety of biomolecules including protein conjugates, antibodies, enzymes, nucleic acids, small molecules, and viruses.

The reagent of interest is mixed at a 1:1 ratio with BioFix™ Buffer, applied to a porous BioFix™ Insert, and dried under vacuum for 30 minutes.

RESULTS

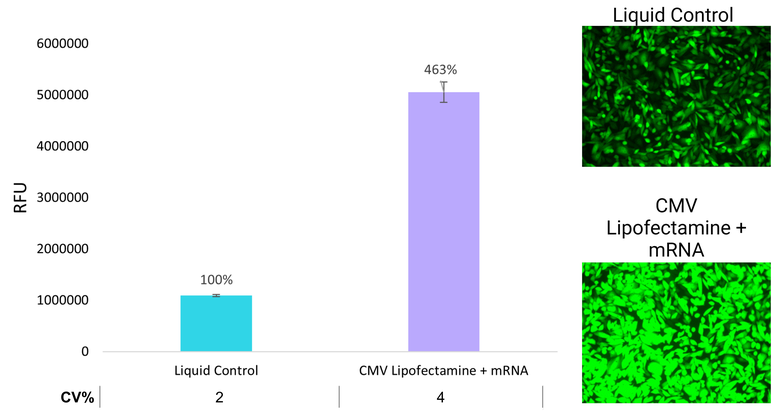
CMV of Lipofectamine Consistently Increases Transgene Expression





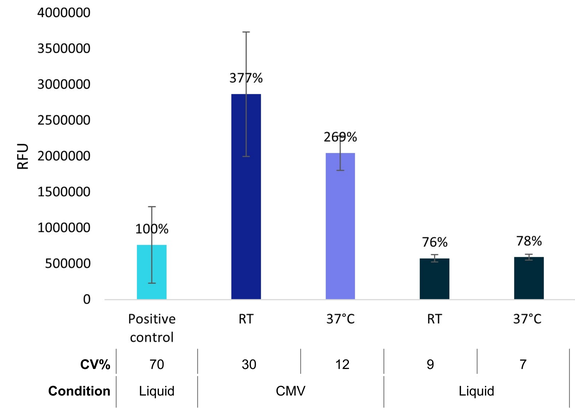
CMV of Lipofectamine MessengerMax. The compatibility of Lipofectamine MesengerMax with the CMV process was evaluated through endpoint fluorescence intensity in CHO cells. After rehydrating lipofectamine from the scaffold, it was combined with mRNA coding for eGFP and used to treat cells. Activity was measured following 24 hours of incubation at 37°C. The Upkara CMV product outperformed the liquid control with a 2.5-fold increase in transgene expression (n=27 ± SD).

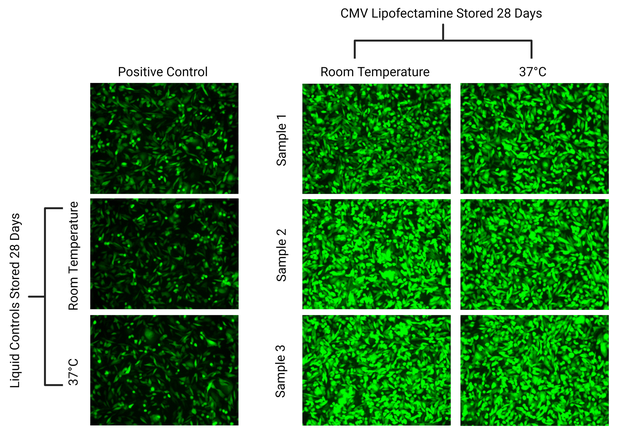
Complexed mRNA-Lipofectamine is Compatible with CMV



CMV of complexed mRNA-Lipofectamine. Activity of complexed mRNA and lipofectamine MessengerMax was evaluated through endpoint fluorescence intensity in CHO cells. mRNA coding for eGFP was complexed with Lipofectamine MessengerMax, applied to a solid support scaffold, subjected to CMV, rehydrated, and used to treat CHO cells. Activity was measured following 24 hours of incubation at 37°C. The Upkara CMV product outperformed the liquid control with a 4.5-fold increase in transgene expression (n=3 ± SD).

Activity of CMV-Lipofectamine Maintained After Stress Period





Stressed CMV-Lipofectamine MessengerMax activity. The activity of Lipofectamine MessengerMax was evaluated post-CMV under stress conditions by incubating the transfection reagent housed in the solid support scaffold at different temperatures. The stability of CMV-Lipofectamine MesengerMax under stressful temperature conditions was evaluated through endpoint fluorescence intensity in CHO cells. After rehydrating lipofectamine from the scaffold, it was combined with mRNA coding for eGFP and used to treat cells. Activity was measured following 24 hours of incubation at 37°C. The Upkara CMV product outperformed the positive liquid control with a minimum 2.5-fold increase in transgene expression relative to each stress condition (n=3 ± SD).

BIBLIOGRAPHY

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3. Mohanty P, Chakraborty N. Capillary Assisted Vitrification Processes and Devices. United States Patent and Trademark Office; 2020. U.S. Patent U.S. 20200068875March 5.
4. Figures created with BioRender.com

We are actively recruiting collaborators with an interest in exploring our technology.
If Interested, please contact: Jorge Becerra: jbecerra@upkara.com or Laura Bronsart: lbronsart@upkara.com

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