Upkara

Preserving Life,
One Biomolecule at a Time

Development of Thermal Stabile, Ready-to-Use Luminescence Reagents through the Application of Capillary-Mediated Vitrification

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ABSTRACT

Luminescence is a spectrophotometric technique that has been applied to a wide variety of biological and biochemical research applications. Many of the reagents used to generate a luminescence signal, including luciferase, luciferin, and related materials, require cold storage due to their poor thermal stability. Previously, we demonstrated that the thermal stability of luciferase could be improved through the application of Capillary-Mediated Vitrification (CMV), a novel stabilization process. In this report we demonstrate that the same process can be used to improve the stability of luciferin, luciferase/luciferin mastermixes, and the reagents contained in the Lumit®(t) immunoassay kit. We further demonstrate that the CMV-stabilized reagents offer comparable performance to traditional frozen reagents and can be used in a wide range of applications including viable cell counting, gene expression studies, and immunoassays.

INTRODUCTION

Current distribution and storage methods for reagents are inefficient and costly

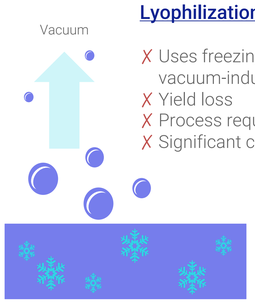
Waste
- ✗ Concentrated formats
- ✗ Dilution waste
- ✗ Limited shelf life

Efficiency
- ✗ Time intensive
- ✗ Risk of error
- ✗ Reagent defrosting
- ✗ Documentation

Storage
- ✗ Expensive
- ✗ Unsustainable
- ✗ Deviation risk
- ✗ Material loss risk

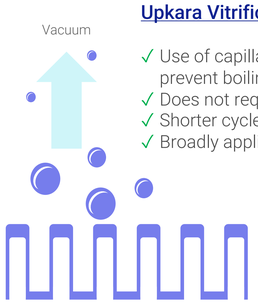
Capillary-mediated vitrification (CMV) is a novel method that provides significant biomolecule stabilization, while being less resource intensive than lyophilization

Lyophilization



- ✗ Uses freezing to prevent vacuum-induced boiling
- ✗ Yield loss
- ✗ Process requires days
- ✗ Significant capex

Upkara Vitrification



- ✓ Use of capillaries to prevent boiling
- ✓ Does not require freezing
- ✓ Shorter cycle times
- ✓ Broadly applicable

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 freezing (1-4).

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6. Figures created with BioRender.com

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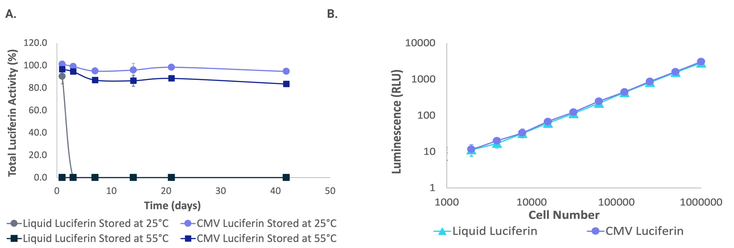
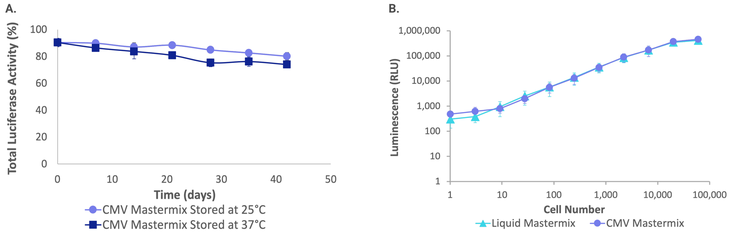
RESULTS

Stabilized Luciferin



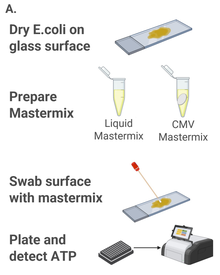
CMV-Stabilized Luciferin. **(A)**The performance of CMV-stabilized luciferin and liquid-control luciferin following thermal stress at 25°C and 55 °C was assessed over 42 days. Activity was measured using an in-house ATP assay. CMV samples had nearly equivalent activity as the frozen control while stressed-liquid samples lost all activity within 3 days (n=3 ± SD). **(B)** As luciferin is heavily used to quantify luciferase-expressing cells *in vitro* and *in vivo*, we next evaluated the performance of CMV-stabilized luciferin for quantifying a luciferase-expressing 4T1 murine mammary cancer cell *in vitro*. The CMV-stabilized luciferin performed equivalent to frozen luciferin for quantification of luciferase-expressing cells (n=3 ± SD).

Stabilized Luciferase-Luciferin Mastermix



CMV-Stabilized Luciferin-Luciferase Mastermix. Although luciferin is frequently used to quantify luciferase-expressing cells, it can also be used to quantify non-luciferase expressing cells by lysing them and adding exogenous luciferase. **(A)** The performance of a CMV-stabilized luciferin-luciferase mastermix was assessed following thermal stress at 25°C and 37 °C over 42 days. Activity was measured using an in-house ATP assay. The CMV-stabilized samples had nearly 80% activity of a frozen control following 42 days of stress (n=3 ± SD). **(B)** Furthermore, we assessed the CMV-stabilized luciferin-luciferase mastermix performance for quantifying 4T1 murine mammary cancer cells that did not express luciferase *in vitro*. The CMV-stabilized luciferin-luciferase mastermix performed equivalent to the freshly prepared liquid control (n=3 ± SD).

Stabilized Luciferase-Luciferin-Lysis Buffer Mastermix



B.

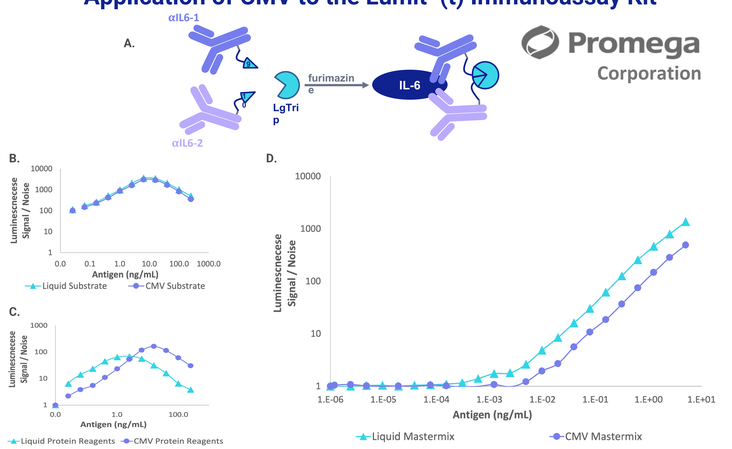
Liquid Mastermix			CMV Mastermix		
RLU	Identity	Accurate	RLU	Identity	Accurate
601	negative	✓ TRUE	1267	positive	✓ TRUE
1613	positive	✓ TRUE	1345	positive	✓ TRUE
503	negative	✓ TRUE	756	negative	✓ TRUE
517	negative	✓ TRUE	1126	positive	✓ TRUE
1193	positive	✓ TRUE	575	negative	✓ TRUE
1217	positive	✓ TRUE	1161	positive	✓ TRUE
415	negative	✓ TRUE	435	negative	✓ TRUE

CMV-Stabilized Luciferin-Luciferase-Lysis Buffer Mastermix. In addition to quantifying mammalian cell lines for research applications, the luciferase-luciferin ATP assay is used to detect contamination in food and clinical settings. **(A)** The performance of a CMV-stabilized luciferin-luciferase-lysis buffer mastermix was assessed for its ability to detect bacterial contamination. E.coli was air dried on a glass slide. A swab was moistened in either a freshly prepared mastermix (Liquid) or a CMV-stabilized mastermix that had been reconstituted (CMV). The moistened swab was used to collect a sample from the contaminated glass surface and returned to the corresponding mastermix preparations. **(B)** In a blind study, an analyst evaluated 7 surfaces using the liquid mastermix or the CMV-stabilized mastermix. Both formats performed equally well in defining whether a surface was contaminated or not. For this proof-of-concept, the limit-of-detection was 1x10^5 bacteria per dried surface *(data not shown)*.

We are actively recruiting collaborators with an interest in exploring our technology.
If interested, please contact: Mary Shank-Retzlaff: mretzlaff@upkara.com or Laura Bronsart: lbronsart@upkara.com

RESULTS

Application of CMV to the Lumit®(t) Immunoassay Kit



CMV Stabilized Lumit®(t) Assay. Promega Corporation is exploring novel ways to leverage the high-sensitivity of luciferase-based assays to improve antigen quantification. (A) Schematic of Promega's *ternary* NanoLuc (NanoTrip) reporter immunoassay. Through Promega's previously established direct-label methodology, β9 and β10 peptides are chemically conjugated to a pair of antibodies that recognize separate epitopes on the target analyte. Binding to the analyte brings the antibodies, and their respective peptide subunits, into close proximity. With the addition of LgTrip and the furimazine substrate, complementation of the active ternary Nluc complex occurs, producing bioluminescence (5). In collaboration with Upkara, Promega evaluated whether the Upkara-technology could be used to create a mastermix format of the Lumit®(t) assay. **(B)** The furimazine substrate was evaluated for performance in an antigen-detection assay. The CMV-treated furimazine performed identical to the liquid control substrate (n = 2). **(C)** Promega assessed how CMV-treatment impacted the performance of a protein mastermix consisting of the β9 and β10 peptide-conjugated pair of antibodies and the LgTrip relative to liquid control reagents. The CMV-treated mastermix did show a slight shift in sensitivity (n=2). **(D)** Furthermore, the performance of an all-in-one mastermix consisting of the furimazine, the β9 and β10 peptide-conjugated pair of antibodies, and the LgTrip was assessed. Similar to the protein mastermix alone, a slight shift in sensitivity was observed following CMV treatment. It is anticipated the sensitivity can be recovered with additional optimization.

CMV PROCESS and USE

CMV Reagent Manufacturing

The reagent of interest is diluted to an appropriate concentration, mixed at a 1:1 ratio with BioFix™ Buffer, applied to a solid, porous support referred to as a BioFix™ scaffold, and processed within a chamber for 30 minutes.

CONCLUSIONS

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. Furthermore, the process is amendable to complex reagent mixtures whereas lyophilization often requires a specific formulation and cycle process for each unique component. This aspect of the CMV technology lends itself to the development of mastermixes. Such mastermix kits enhance the customer experience by improving the ease-of-use and efficiency. Furthermore, if the kits can be stored and shipped ambiently, it improves both manufacturer and customer sustainability while reducing risk of reagent loss due to temperature excursions.