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Preserving Life,
One Biomolecule at a Time

Capillary-Mediated Vitrification: A Novel Approach for Preparing Thermostable, Ready-to-Use Reagents

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ABSTRACT

Currently, biological reagents require temperature-controlled storage and distribution, which is both energy and resource intensive. Additionally, reagents are supplied at high concentrations and volumes resulting in significant material discard and complicated dilution schemes. We have developed a bio-preservation method, capillary-mediated vitrification (CMV), that enables storage of reagents at ambient temperatures and at concentrations designed to match the assay requirements.

In this report, we demonstrate the stability and performance of CMV-stabilized reagents. CMV is a promising alternative to traditional biopreservation methods that significantly improves analytical workflow efficiencies and eliminates the need for cold storage.

INTRODUCTION

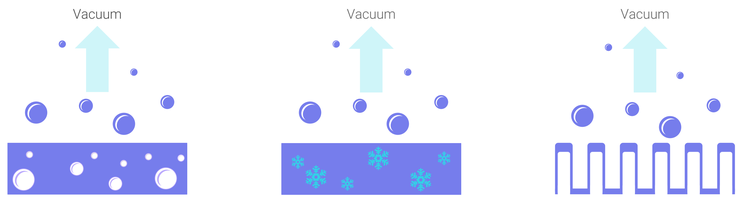
Current distribution and storage methods for reagents are inefficient and costly

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



Vacuum-Based Desiccation
- ✗The use of a vacuum to cause desiccation results in boiling

Lyophilization
- ✗Freezes to prevent boiling
- ✗Results yield loss
- ✗Process requires days
- ✗Significant capex

Upkara Vitrification
- ✓Uses capillary evaporation
- ✓Prevents 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 a freezing step (1-3).

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.

*Figures created with BioRender.com

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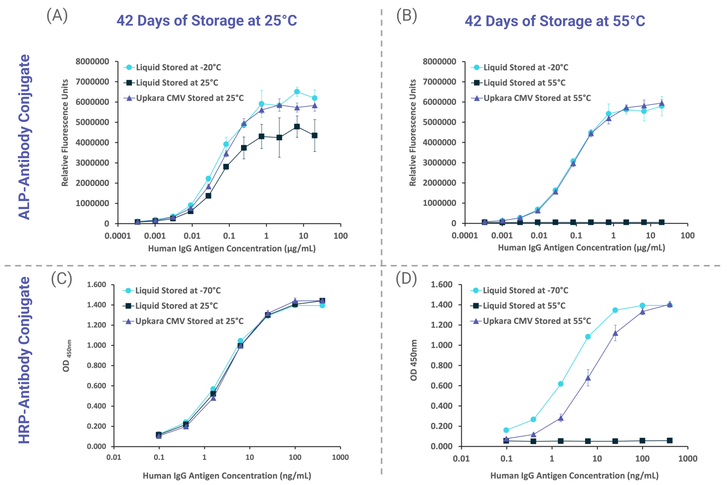
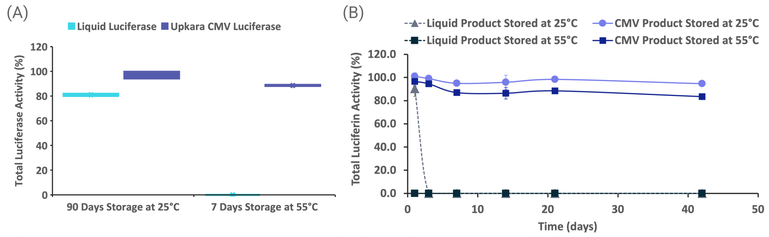
RESULTS

Stability of CMV-Stabilized Antibody-Conjugates



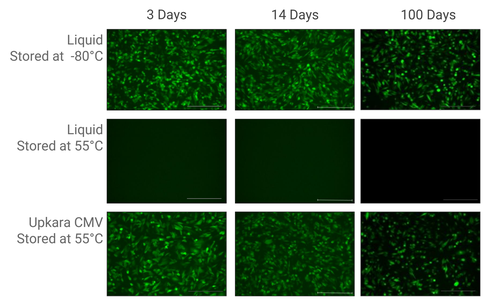
CMV preserved enzyme-antibody conjugates. The performance of stabilized and control ALP (A, B) and HRP (C, D) antibody conjugates was evaluated via ELISA. Activity was measured following 42-days of storage at 25°C and 55°C. Reagents performed similarly to frozen controls. At 55°C a small shift in ED50 was seen for the HRP conjugate, but a full dose response was obtained (n=3 ± SD).

Stability of CMV-Stabilized Luciferase and Luciferin



CMV preserved luciferase and luciferin. Activity of stabilized and control samples was measured via microplate reader. (A) Total luciferase activity following 90 days of storage at 25°C and 7 days of storage at 55°C; (B) Total luciferin activity following 42 days of storage at 25°C and 55°C. Percentage of total activity relative to the frozen controls is graphed (n=3 ± SD).

Stability of CMV-Stabilized GFP-mRNA



CMV preserved green fluorescent protein encoding mRNA (GFP-mRNA). CHO-K1 cells were transfected using CMV preserved and liquid control GFP-mRNA following storage at -80°C or 55 ° C. (n=3).

CMV PROCESS and USE

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.

CMV Reagent Manufacturing

Step 1
Solution of buffer and reagent is mixed

Step 2
Mixture is dispensed

Step 3
Inserts are stabilized using a chamber

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 as part of a BioFix™ Insert, and dried under vacuum for 30 minutes.

Current Reagent Paradigm

Step 1
Distribution and storage at cold temperatures

Step 2
Reagents are defrosted before use

Step 3
Reagents are serially diluted

Step 4
Reagents are used in assays

For a single assay, reagents often require different storage conditions and unique preparations. These aspects increase the resources needed to conduct experiments and can result in assay errors.

Upkara Reagent Paradigm

CMV-stabilized reagents can all be stored under ambient conditions and can be packaged in quantities that match the assay needs. This results in not only a convenient storage condition, but improved assay performance and a reduced need for experimental repeats.

Step 1
Distribution and storage at ambient

Step 2
Reagent is eluted from scaffold

Step 3
Reagents are used in assays

CONCLUSIONS

Reagent Waste
- ✓ Assay-specific designs
- ✓ No dilution steps
- ✓ Extended shelf life

Workflow Efficiencies
- ✓ Simple testing workflows
- ✓ Reduced error
- ✓ Improved performance

Storage Requirements
- ✓ No cold storage
- ✓ No cold shipment
- ✓ No deviation risk
- ✓ Reduced complexity

Capillary-mediated vitrification (CMV) is a novel, simple and easily-optimized process that enables ambient shipping and storage of a variety of biomolecules. Application of this technology can reduce operational costs, expand market access, and improve an organization's environmental sustainability practices.