A Room Temperature Metabolism Quenching Method for Automated Metabolomics Sample Preparation

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Introduction

Many existing methods for cell-based metabolomic sample preparation require low-temperature metabolism quenching procedures, which prevent interconversion of metabolites in a rapid, controlled manner. The low temperatures required to quench metabolism often utilize cold liquids, such as liquid nitrogen, which are difficult to handle and store. Additionally, low temperatures complicate automation of metabolism quenching steps, since they promote collection of condensation on robotic components. This condensation can hinder robotic movement and cause rusting. We developed a mass-spectrometry-compatible method for room temperature metabolism quenching and simultaneous cell lysis to avoid the issues associated with low temperature quenching.

Advantages of Room Temperature Lysis and Quenching:

- Room temperature workflow is simpler and safer than traditional workflows
- Room temperature lysis and quenching are more easily automated than traditional workflows that utilize hot or cold quenching solutions
- Includes the integration of the Agilent Captiva EMR-Lipid plate which captures lipids for improved LC/MS performance

Workflow

1. Count and Harvest Cells
2. Wash Cells (optional)
3. Lyse Cells and Quench Metabolism at Room Temperature (RT Solution)
4. Precipitate Proteins
5. Adjust Solvent Composition
6. Sample Cleanup to Remove Lipids
7. Dry Samples
8. Reconstitute Samples
9. Analyze Samples by LC/MS

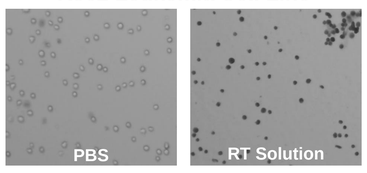
Conclusions

- We developed a room temperature method for mammalian cell lysis and metabolism quenching that is more amenable to automation than conventional methods.
- We optimized conditions for clean-up of cell lysates using the Captiva EMR-lipid sorbent.
- Initial comparison shows similar numbers of peaks are detected in samples prepared with our room temperature method compared to a traditional method.
- Work is ongoing to automate the room temperature method on the Bravo liquid handling platform and quantify performance compared to traditional methods.

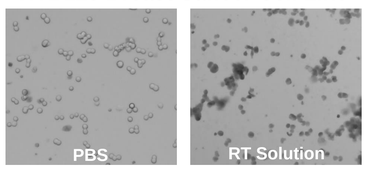
3. Lyse Cells and Quench Metabolism at Room Temperature (RT Solution)

Room Temperature Cell Lysis

K562 Leukemia Cell Line

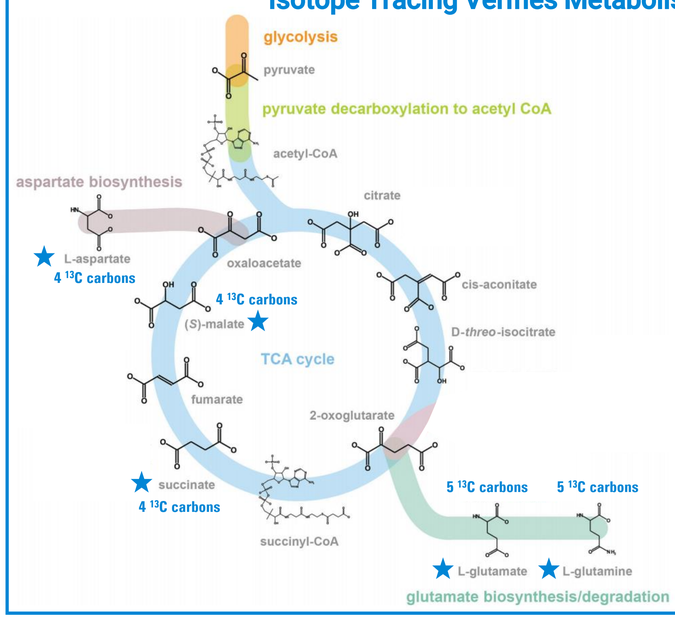


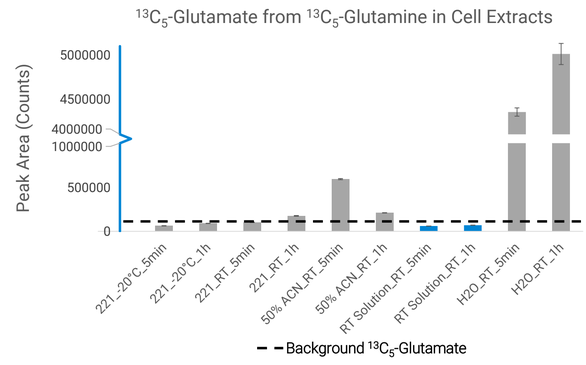
HTB123 Breast Cancer Cell Line



A single solution (RT solution) was used for room temperature cell lysis and metabolism quenching. Trypan blue staining indicates the RT solution lyses mammalian suspension cells. Brightfield images show cells not lysed (not stained) or lysed (stained).

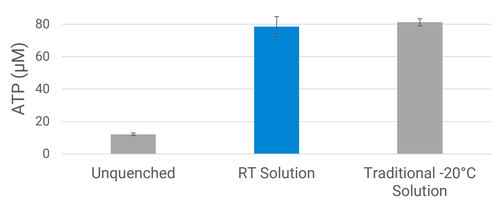
Isotope Tracing Verifies Metabolism Quenching at Room Temperature





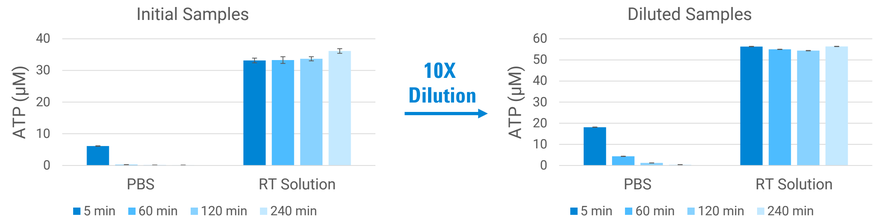
Metabolism quenching was monitored in K562 cell lysate using isotope tracing with $^{13}C_5$-glutamine. Left: schematic of the TCA cycle, indicating the flow of carbon-13 atoms as they enter the cycle, downstream of $^{13}C_5$-glutamine. ★ indicates compounds monitored. Above: $^{13}C_5$-glutamate detected in cell lysate from cells lysed and quenched with solutions containing $^{13}C_5$-glutamine. The RT solution performs better than all other lysis and quenching solutions, including room temperature 2:2:1 MeOH:ACN:H_2O, 0.1M FA (221).[1] RT = room temperature.

Room Temperature Solution Extracts ATP



Equivalent amounts of ATP were extracted from K562 cells by the RT solution at room temperature and a traditional extraction solution, 2:2:1 MeOH:ACN:H_2O, 0.1% FA, at -20°C.[1] Lysed, unquenched cells exhibit lower ATP levels.

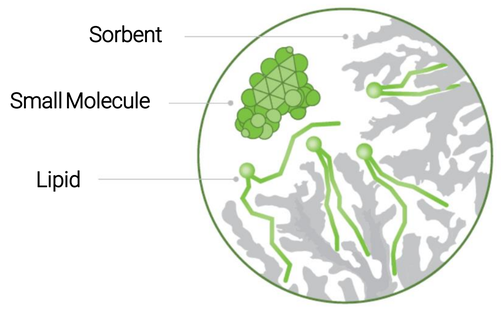
ATP is Stable in Cell Lysates Generated with RT Solution



ATP extracted with the RT solution is stable at room temperature for up to four hours, while ATP extracted into PBS via probe sonication is not stable. ATP extracted with the RT solution remains stable at room temperature after a 10-fold dilution into PBS. For diluted samples, the measured ATP concentration was multiplied 10-fold.

6. Sample Cleanup to Remove Lipids

Captiva EMR-Lipid for Lipid Removal

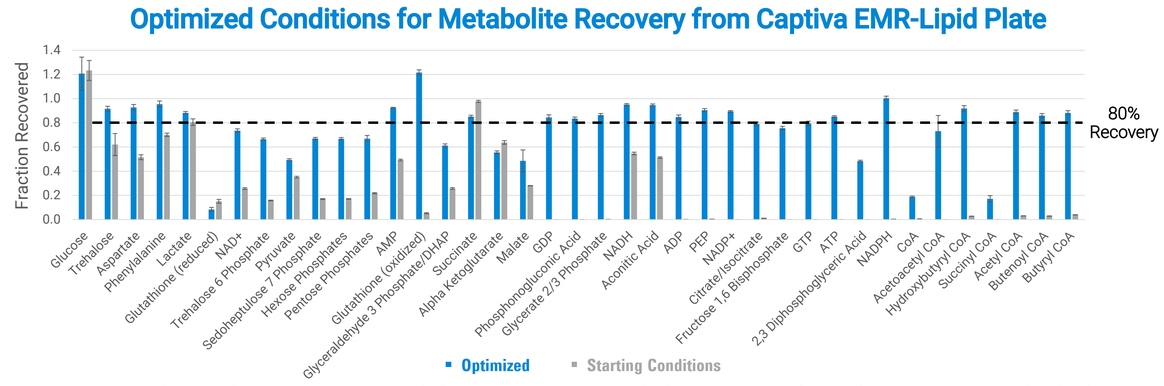


Lipid removal improves LC/MS performance by reducing matrix effects caused by phospholipids[2]. The Captiva EMR-lipid (Enhanced Matrix Removal) sorbent removes lipids based on size exclusion and hydrophobic interactions.

96-Well Plate Format — Compatible with Bravo Platform

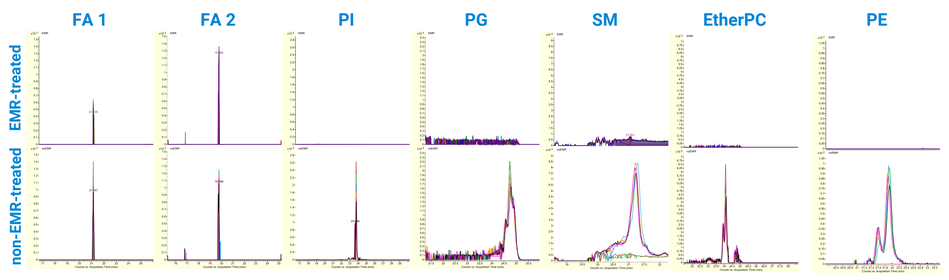
Captiva EMR-lipid 96-well plates have been utilized in the Bravo Metabolomics Sample Prep Platform, which automates metabolomics sample preparation for plasma samples.

Optimized Conditions for Metabolite Recovery from Captiva EMR-Lipid Plate



Optimized sample conditions were created for maximizing metabolite recovery from the Captiva EMR-lipid plate. The optimized conditions have an average 80% recovery of central carbon and other metabolites, as measured by LC/MS.[3] Similar metabolite recoveries are obtained with cell lysates made using the RT solution.

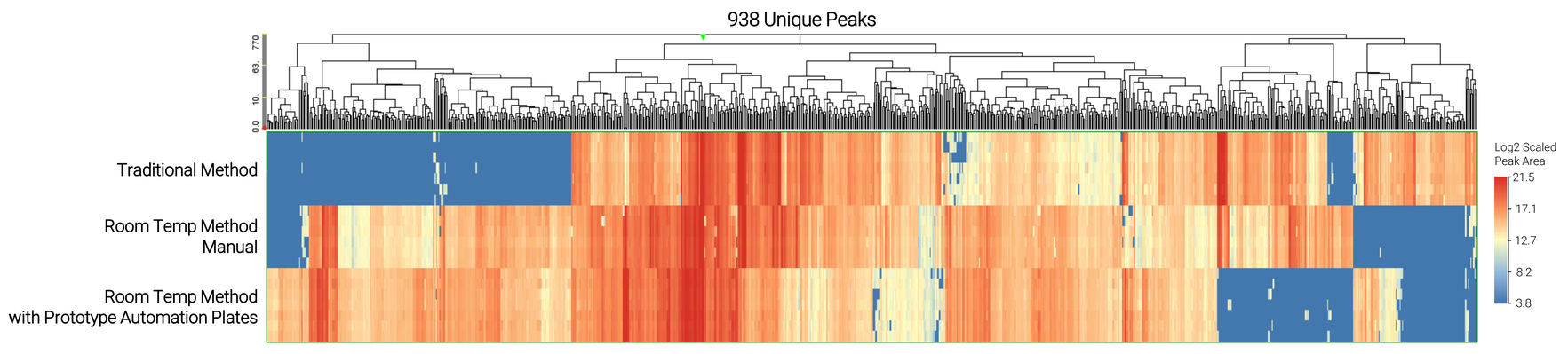
Captiva EMR-Lipid Plate Removes Lipids under Optimized Metabolite Recovery Conditions



Captiva EMR-lipid plate removes lipids from cell lysate samples prepared using the RT solution and conditions optimized for metabolite recovery from the Captiva EMR-lipid plate. Fatty acids are incompletely removed while PI, PG, SM, PC, and PE lipids are completely removed from the cell lysate samples.

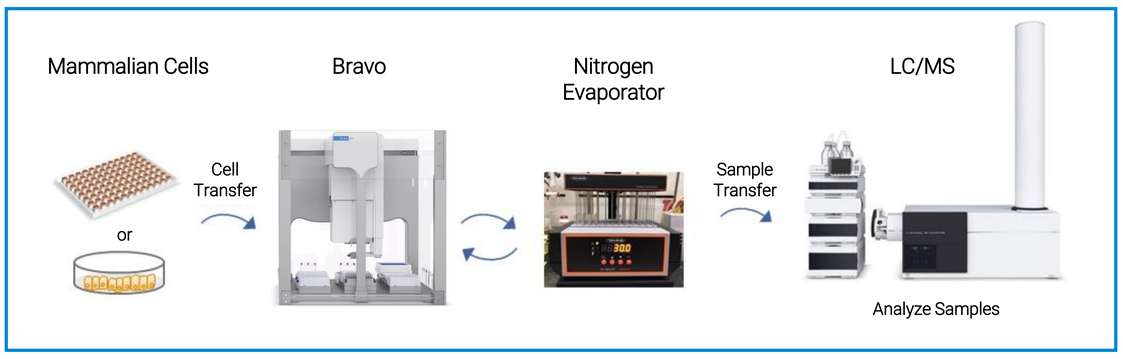
9. Analyze Samples by LC/MS

Preliminary Untargeted LC/MS Comparison of the Room Temperature Workflow to a Traditional Workflow



Samples were prepared from K562 cells using either the developed room temperature workflow (manual or with plates from a prototype automated workflow) or a traditional workflow utilizing -20°C temperatures. Cell samples were analyzed by LC/MS.[3] Data analysis was completed using Agilent Profinder and Mass Profiler Professional software. All peaks present in significant levels in blank injection samples were removed from the data. A similar number of peaks are found using all methods. Differences between the methods are under investigation.

Planned Room Temperature Automated Workflow



References

1) Lu, W., Su, X., Klein, M.S., Lewis, I.A., Fiehn, O., and Rabinowitz, J.D. Metabolite Measurement: Pitfalls to Avoid and Practices to Follow. *Annu. Rev. Biochem.* 2017, *86*, 277-304.

2) Zhao, L. and Lucas, D. Efficiency of Biological Fluid Matrix Removal Using Agilent Captiva EMR-Lipid Cleanup. *Agilent Technologies Application Note.*

3) Hartman, T. E., Rhee, K. Y., Dai, Y. Metabolomics Analysis of Tuberculosis Drug Activity Using an Agilent 6545 Q-TOF LC/MS. *Agilent Technologies Application Note.*

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