

Solving the HTS Compound-Thawing Bottleneck

by Kurt O. Lund

Many investigators are familiar with the operations of high-throughput screening (HTS), in which various chemical and biological compounds are sampled, read, and processed automatically with the use of computer-controlled robots and readers, etc. However, before any of this automation can take place, it is necessary for the compounds to be removed from the freezer and for them to be thawed into a liquid state for sampling. In terms of laboratory efficiency, this thawing requirement is an enormous bottleneck.¹

Although alternatives to freezer storage are under development,² the preferred method of compound preservation is to freeze them to a subzero temperature (e.g., -20 to -80 °C) in (deep) 96-well microplates, such as that shown in Figure 1. Pharmaceutical and biotechnology companies have large and costly libraries of such plates of compounds. Not only is ordinary thawing itself time consuming and inefficient, it is also wasteful. Thus, if compounds are only needed from one or two wells (as is frequently the case), then the entire plate must first be thawed. The remaining, nonsampled compounds degrade upon refreezing, severely overstressing compound libraries.³

This problem is so important that it has led to the introduction of trays of individually removable wells.^{4,5} Essentially, this practice returns the storage and retrieval processes to the days of test tubes and requires individual tracking of the tubes. To address this issue, a miniature thermal control unit (TCU) was developed that can rapidly thaw an individually selected well without affecting the other frozen wells in the plate (Rapid Thaw System [RTS],¹ ACESystems, Del Mar, CA).

Benchtop thawing

The traditional way to thaw a microplate is to place it on the laboratory bench and wait. To illustrate this thawing bottleneck, the deep-well microplate in Figure 1 was filled with filtered water (and subsequently with dimethylsulfoxide [DMSO]), and fitted with two K-type thermocouples that extend approximately halfway down in a center well and a side well, as shown. The filled microplate was placed overnight in a conventional food freezer having a temperature of approx. -10 °C, with the two thermocouples frozen in

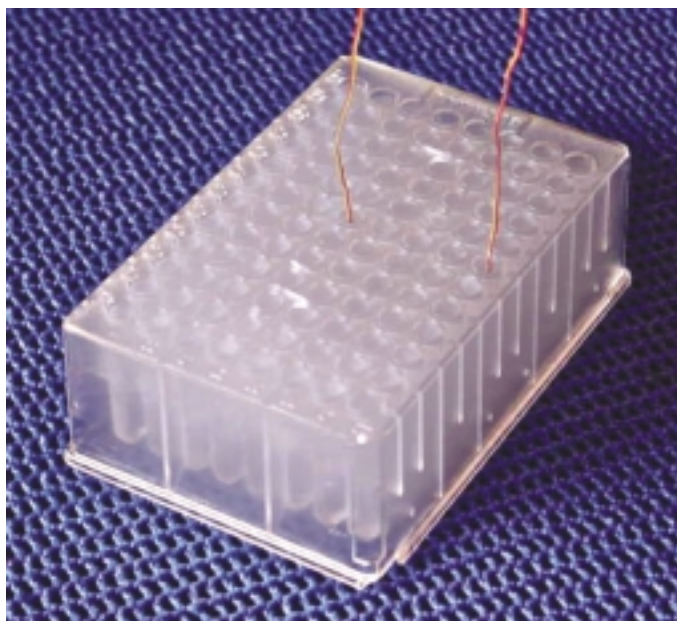


Figure 1 Deep-well microplate with thermocouples.

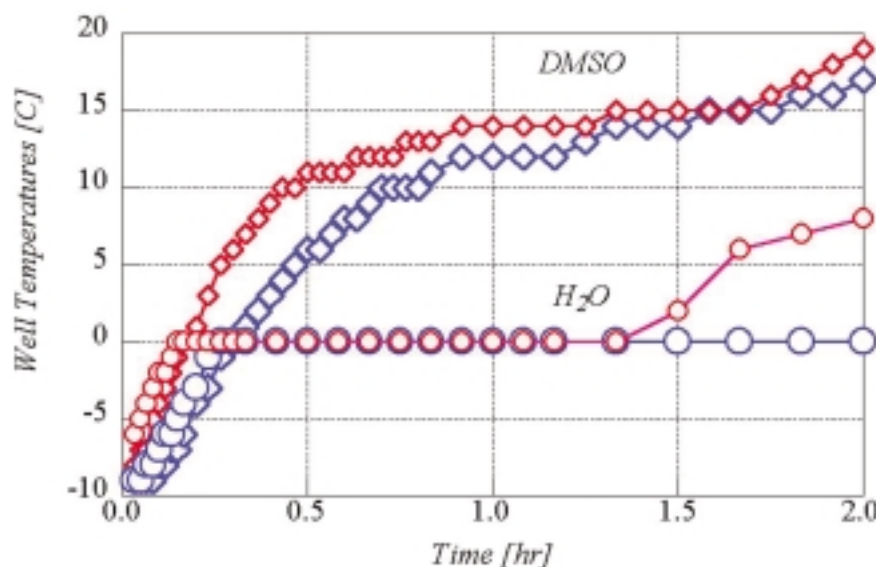


Figure 2 Well temperature profiles.

place. The plate was then removed from the freezer, placed on a laboratory bench, and allowed to thaw. The ambient room temperature varied from 24 °C at the start to 26 °C at the end for the test with water, and from 27 °C to 29 °C for the test with DMSO. The results are shown in Figure 2; the symbols in blue indicate data for the center-well thermocouple, and those in red are for the side-well thermocouple. The circles refer to the test with water, and the diamonds to DMSO.

For the water test, it can be seen that the two wells with thermocouples remain at the thawing temperature (0 °C) for several hours, which

means that the wells stayed partially frozen for this time period (i.e., the benchtop heat transfer is very poor). Thus, the side well was not fully thawed until approx. 1.5 hr after the start, when its temperature begins to increase above zero, as seen from the red circles in Figure 2. The central well remained partially frozen at 0 °C, even after 3 hr. Indeed, removal of the center thermocouple at 2 hr showed that it was covered with ice approx. 5 mm in diameter and 15 mm in length, indicating less than 50% thawing, and this is starting with the ice in the freezer at only -10 °C.

For the DMSO test, incipient thawing did not begin until approx. 40 min, when the center-well thermocouple in Figure 2 read +10 °C. Thawing progressed slowly until after 2 hr, when the thermocouple was entirely in liquid and read +17 °C. However, even at this time, there was frozen DMSO in the bottom quarter of the well (solid DMSO is more dense than its liquid and thus remains at the bottom unless stirred). Hence, for DMSO as well as for water solutions, an inordinate amount of time is required for thawing on the bench.

Heat-up and onset of thawing

In addition to the time taken for the actual, latent heat thawing, there is a heat-up time required to reach the onset of thawing. Starting at approx. -10 °C, it can be seen in Figure 2 that for water this takes approx. 15 min for the center well and approx. 7 min for the side well. With samples frozen to lower temperatures (e.g., -20 to -80 °C), the time to reach the onset of thawing would be correspondingly longer, as can be estimated as follows:

In simplest terms, the initial heat transfer from the ambient air to the ice (or frozen compound) constitutes a resistance-capacitance (RC) circuit,⁶ which has the temperature increase equation:

$$T_w = T_a - (T_a - T_0) e^{-t/RC}$$

where T_w , T_a , and T_0 are the well, ambient (room), and initial (freezer) temperatures, respectively; R is the thermal resistance; C is the capacitance; and the product, RC , is the time constant of the heat-up process. If the equation is inverted, the time, t_m , for T_w to reach the melting temperature, T_m , is:

$$t_m = RC \ln \left\{ \frac{T_a - T_0}{T_a - T_m} \right\}$$

A comparison of this equation with the heat-up rates for water in Figure 2 shows time constants of $RC \approx 60$ min for the center wells and ≈ 40 min for the side wells.

These time constants are basically invariant for all initial temperatures, T_0 ; thus, the estimated time to reach the onset of water thawing was determined (Figure 3). If the plate remains in the freezer at -80°C , it is estimated that thawing in a central well would not start before 1.5 hr after placing the plate on the bench, and in a side well not before 1 hr, and so on for other freezer temperatures. Similar estimations can be made for DMSO. Therefore, at lower freezer temperatures, the time for thawing would be even greater than those measured.

Nevertheless, regardless of the initial freezer conditions, and of solvent types, it takes several hours to thaw a microplate on the bench. The thawing can be accelerated somewhat by placing (actually floating) the microplate in a circulation bath; however, this is not only messy and inconvenient, but still takes an extraordinary amount of laboratory time (>1 hr), and the nonselected compounds are still wasted.

Thermal control unit

For all deep circular-shaped 96-well microplates, the RTS utilizes a tiny TCU for each well, which rapidly thaws the well contents in a safe and gentle manner. This not only removes the thawing bottleneck and saves valuable laboratory time, but also preserves precious compounds by thawing only those wells to be sampled. One such TCU design is shown in Figure 4. In the RTS, the TCUs would be thermally controlled.

Each TCU is designed to fit snugly around a standard deep circular well of a 96-well plate, as shown in the cutaway microplate in Figure 5. By electronically controlling the TCU to a pre-selected temperature (e.g., 30°C), the compound in that well can be safely maintained below this temperature, and yet have a thawing heat source available immediately surrounding it. System details are given in Ref. 1.

Feedback-controlled thawing

To illustrate the benefits of controlled thawing with the TCU, an experiment was conducted on a single well with DMSO, fitted with a TCU; a thermocouple was also

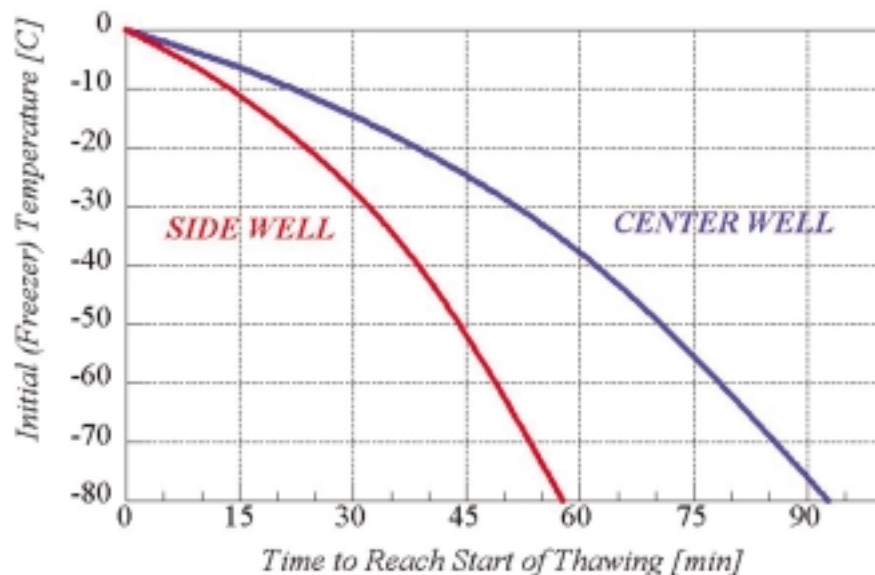


Figure 3 Time to initiate thawing for water.

frozen in place in the center of the well, as described previously. The TCU was equipped with a resistance heater and a thermocouple for computer feedback control, including a standard controller algorithm.

The frozen well with DMSO was removed from the freezer and allowed to equilibrate for approx. 1 min to approx. $+6^\circ\text{C}$ with the room-temperature TCU. Then, at time zero, the command was given to thaw the well, yielding the results shown in Figure 6. The black line, the setpoint temperature, represents the maximum allowable temperature of 30°C . Since the TCU is initially at only $+6^\circ\text{C}$, the feedback control calls for heat input from the heater, thus causing the temperature of the TCU to increase, as shown by the red curve in Figure 6. Because of the thermal mass of the system, the TCU slightly lags behind the command, and somewhat overshoots before attaining 30°C in approx. 1 min. For the center, frozen-in thermocouple shown in blue, there is only a slight increase in temperature, due to the onset of thawing only at the well surface.

Thawing proceeds in this manner until the thaw front reaches the center of the well. Since at 3.5

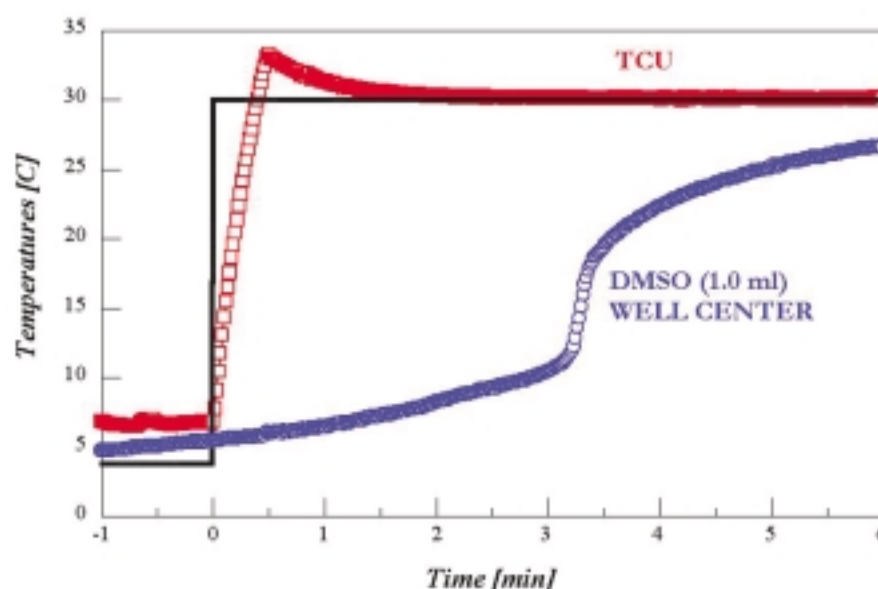


Figure 6 Thawing of DMSO with the TCU.



Figure 4 TCU design.



Figure 5 Cutaway microplate with engaged TCU.

min there is now liquid surrounding the center thermocouple, its temperature increases rapidly, as seen in Figure 6. That is to say, the well thaws 100% in only 3.5 min from the start of command. This is in contrast to the 3.5 hr needed on the bench. Starting from a lower freezer temperature, the TCU thawing takes longer as well, but only minutes longer, not hours. After thawing, the liquid solution temperature approaches that of the controlled TCU, and remains at this temperature indefinitely for sampling, or until the heater is turned off.

Conclusion

The thawing behavior of solvents frozen in microplates was investigated in two types of experiments: 1) conventional thawing on the bench, and 2) rapid thawing with a thermal control unit. The clearest observation from the bench tests is that samples in wells remain mostly frozen for hours after removal from the freezer. Thus, for a

A P P L I C A T I O N N O T E

center well, even 3 hr was not enough to bring about 100% thawing on the bench. The rapid thaw experiment was conducted using computer feedback control of the TCU, which was set to 30 °C. For DMSO in a standard 1-mL well frozen to -5 °C, but starting at approx. +6 °C equilibration, 100% thawing was achieved in only 3.5 min, without an excessive solution temperature.

A standard RC model was fitted to the frozen state data, yielding RC time constants for the initial rate of heat transfer that are in good agreement with heat transfer theory. With the model and time constants, the time for onset of

thawing was estimated for various initial (freezer) temperatures.

Overall, it can be concluded that the RTS significantly enhances laboratory efficiency. In addition, important savings in compound preservation can be realized by thawing only those wells in the microplates that are selected for sampling.

References

1. Lund KO, Theriault Y. Thawing station. U.S. patent #6,106,784, issued Aug 2000. Assigned to ACESystems, Inc., Del Mar, CA.

2. Somers T. GenVault becoming a Fort Knox of DNA: firm streamlines storage, mining of genetic material. *San Diego Union/Tribune* interview with GenVault, Inc., Carlsbad, CA, Jul 29, 2003.
3. Zaayenga A. New hope for overstressed compound libraries. *Am Biotech Lab* 2002; 20(5):44.
4. BD Falcon tube rack storage system. *Drug Discovery and Development*, Jan/Feb 2000.
5. www.abgene.com/product. ABGene, Ltd., Sep 2003.
6. Incropera FP, DeWitt DP. *Fundamentals of heat and mass transfer*. 4th ed. New York: Wiley & Sons, 1996.

Dr. Lund is President, **ACESystems (Applied Chemical & Engineering Systems), Inc.**, 135 Sixth St., Del Mar, CA 92014, U.S.A.; tel.: 858-481-8914; fax: 858-793-2446; e-mail: klund@acesystems.com; home page: www.acesystems.com.