invitrogen

Maintaining Even Temperature Distribution over Chilled Samples in 96-Well Plates and Micro-Centrifuge Tubes

Kenneth D. Lefton, ThermoFisher Scientific, 29851 Willow Creek Rd, Eugene, OR, USA, 97402

ABSTRACT

The speed of chemical reactions is largely dependent on temperature. In cell biology, researchers are often most interested in maintaining the sample above freezing but below roughly 15 degrees C. Often researchers refrigerate their samples or put them on ice just prior to processing and rely on the thermal inertia of the sample and vessel to maintain temperature, since not all processes are housed in a refrigerated environment.

Experimental studies are often set up as a matrix of sample titrations across a well plate. In some cases, the effect of temperature variation across the plate is a significant disturbance in the data set. This is particularly problematic when the samples are at a lower temperature than ambient. The most common format for such testing is ANSI/SLAS 1-2004 96 well plates. Another common format is the micro-centrifuge tube which allows larger volumes (usually up to 2ml) to be utilized in an array of samples, useful for automated sample processing.

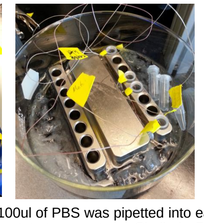
INTRODUCTION

This poster discusses passive cooling solutions for two popular vessel formats, 96 well plate, and micro-centrifuge tubes. For applications requiring the sample to be chilled on ice prior to processing and maintained below 15 deg C, the goal was to minimize sample-to-sample temperature variation across the plate (or array of micro-centrifuge tubes) throughout processing. Additionally, it was determined that the samples need to be maintained below 15 deg C for a minimum of 22 minutes, with a goal of extending this to 45 minutes.

MATERIALS AND METHODS

Common vessel formats; Polystyrene 96 std. round bottom well plate (left), and polypropylene 1.5 & 2ml micro-centrifuge tubes (right).

A Flir SC-300 infared camera was used to view temperature gradients across the plate (or array of micro-centrifuge tubes). Very thin (36 gage) K-type thermocouples were placed in center and corner wells to monitor sample temperature extremes.

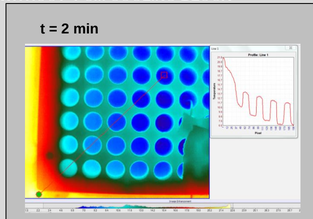


For 96-well plates, 100ul of PBS was pipetted into each well and then placed on ice until reaching steady state temperature. Similarly, 500ul of PBS was pipetted into each micro-centrifuge tube and then placed on ice.
The phase change cooling blocks (one for 96 std round well plates, one for micro-centrifuge tubes) were placed in a refrigerator overnight to freeze the fluid inside and then placed on ice with their respective samples. Samples were ready for test when thermocouple readings bottomed out.

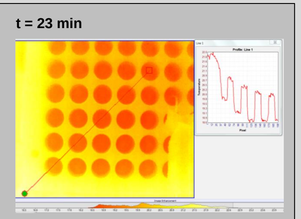
RESULTS - 96 STD. U WELL PLATE

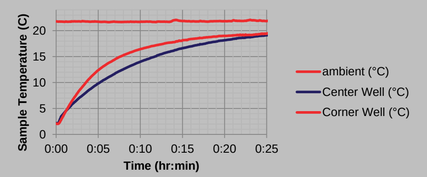
WITHOUT PCM COOLING BLOCK

t = 2 min



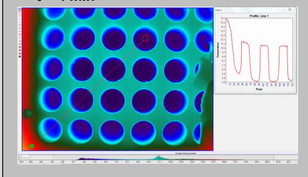
t = 23 min



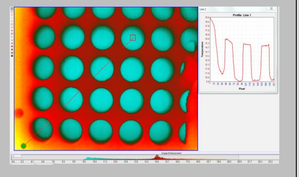


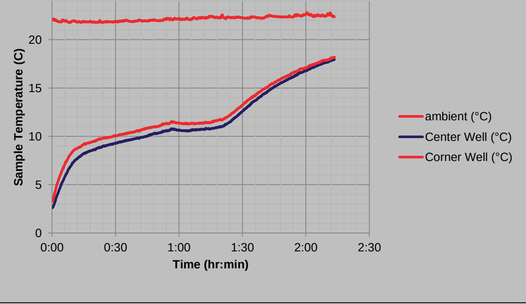
WITH PCM COOLING BLOCK

t = 4 min



t = 45 min

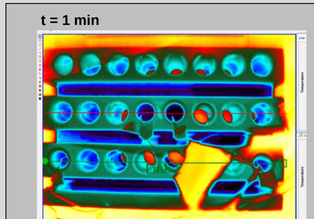




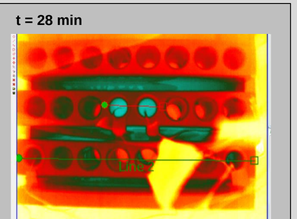
RESULTS - MICROCENTRIFUGE TUBES

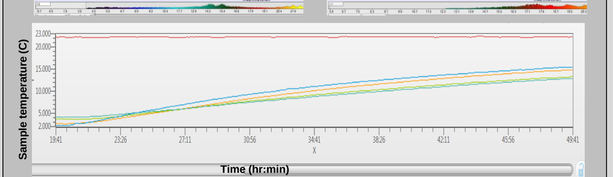
TUBES IN ACETAL HOLDER

t = 1 min



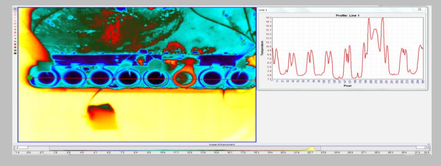
t = 28 min



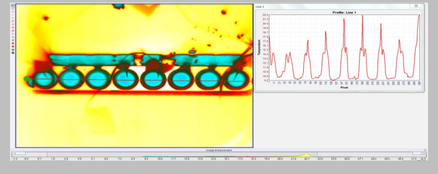


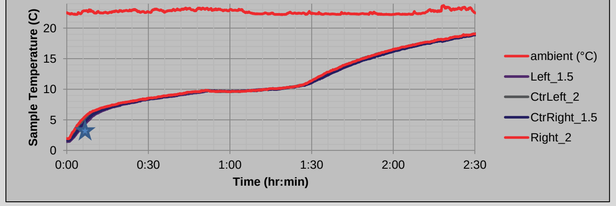
TUBES IN ALUMINUM HOLDER WITH PCM COOLING

t = 1 min

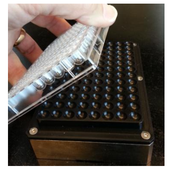


t= 64 min

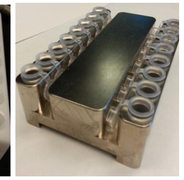




96 Std. well plates on PCM coolingblock



Micro-centrifuge tubes



	96 std Well Plate		Micro-centrifuge Tubes	
	Plate	Plate on PCM cooling block	Tubes in acetal holder	Tubes in alum. holder with PCM cooling
Time to 15degC	7.5 min.	100 min.	27 min.	108 min.
Max sample-to-sample temperature variation	3 C	1-2 C	1-2 C	< 1 C

CONCLUSIONS

The infared imaging largely agrees with the thermocouple data, except there was significant distortion from the fish-eye lens on the IR camera, so only temperatures in the center of the field of view were true. The IR images show that the heat transfer from the acetal tray in direct contact below the cooling block is reasonably low (temperature of the acetal tray is within a couple degrees C of ambient).

The 96-well plate alone (without cooling block) did not meet the 22 minute minimum. The temperature variation from center well to corner well was also larger for the plate alone (about 3 deg C).

The phase-change 96-well and micro-centrifuge tube holder provide the best processing time (over 100 min) and had the smallest sample-to-sample temperature variation (< 1 deg C). The flatter, more horizontal portion of the temperature profile is where the phase-change material was changing from solid to liquid. The plastic micro-centrifuge holder passed the minimum processing time.

Future study could measure different plate materials and/or bottom configurations, or possibly devise a universal solution.

ACKNOWLEDGEMENTS

Ken Lefton, and engineering interns Tyler Vonderach and Spencer Lazaroff

ThermoFisher SCIENTIFIC