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Application Note

BENEFITS OF READERS WITH INTEGRATED COOLING ON ASSAY STABILITY

INTRODUCTION

Temperature is a major factor affecting the rate chemical reactions. The average speed of a reaction is related to the square root of the absolute temperature, as described by the Arrhenius equation:

$$k = Ae^{-E_a/(k_B T)}$$

All instruments, including multimode readers, emit heat from mechanical and electronic components. This heat can affect the reaction kinetics of samples being investigated inside the reader; an increase in temperature of 10 °C can cause a two- to three-fold change in the rate of a biological or chemical reaction. The Spark® multimode reader's ingenious Te-Cool™ module allows the measurement chamber to be set at temperatures between 18 and 42°C. This improved temperature control limits the effect of heat on the dynamics of enzymatic reactions, and can lead to significant differences in the kinetic profiles compared to readers without active cooling.

This application note describes the results of a series of assays investigating the temperature susceptibility of bioluminescent and biofluorescent reactions, kinetic profiling and high throughput screening.

MATERIAL AND METHODS

The following assays were used for the experiments:

- Succinate-Glo™ (Cat.# V7990)
- Kinase-Glo® Assays (Cat.# V6711)
- ApoLive-Glo™ Multiplex Assay (Cat.#G6410)
- CellTiter-Glo® 2.0 Assay (Cat.# G9241)
- UDP-Glo™ Assay (Cat.# V6961)
- ADP-Glo™ Assay (Cat.# V6930)
- GTPase-Glo™ Assay (Cat.# V7681)

For kinetic assay monitoring (Figures 2, 3, 4, and 5), multiple wells of 384 well plates were filled with identical assay solutions, and the luminescence signals were read (integration time = 300 ms). Whole plate luminescence or fluorescence was measured every five minutes, and signals from multiple identical assay wells were averaged.

The LOPAC 1280 library (Sigma Aldrich, # LO4100-1EA), a collection of 1280 pharmacologically-active compounds, was screened in quadruplicate in low-volume, 384-well plates at a final compound concentration of 10 µM.

All assays were performed in white low-volume, round bottom 384-well plates (Corning, #4512) with a reaction volume of 18 µl. Assay reagents where added using a Multidrop™ Combi nL Reagent Dispenser (Thermo Fisher Scientific). Room temperature varied from 19 to 24 °C. The measurement parameters were set according to the instructions for use for each individual assay.

RESULTS AND DISCUSSION

Luminescence read times and the reader's internal temperature and plate read time can have significant impact on the reaction volume temperature and reaction rate.

The luminescence read time per plate depends on the number of wells measured and the read time per well. Typical measurement times for the Spark reader are shown in Figure 1. Relatively long plate read times are sufficient to cause the temperature of the reactions to increase, as the internal temperature of readers that are not actively cooled can be 2-10°C higher than the ambient laboratory temperature, leading to an acceleration of the chemical processes in the assay.

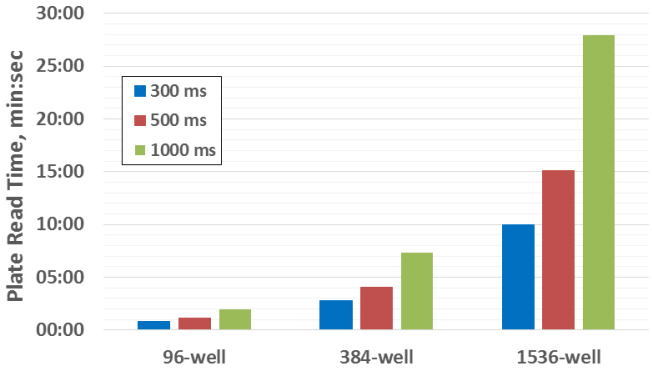

Figure 1: Read times in 96-, 384- and 1,536-well plates using integration times of 300, 500 and 1,000 ms.

Bioluminescent and biofluorescent reaction rates are affected by temperature

Figures 2 and 3 show the effect of temperature on the bioluminescent Succinate-Glo assay and the biofluorescent ApoLive-Glo viability assay. Without cooling, the luminescence signal of the Succinate-Glo assay increases significantly with time, due to the plate and the reaction solutions warming up inside the reader. In comparison, luminescence signals measured in the Spark reader were stable over time, as the Te-Cool module enabled environmental control of the reader chamber,

matching the ambient laboratory temperature (Figure 2). This relative change in the luminescent signal is independent of the concentration of the succinate substrate ([c] = 5 or 1 µM).

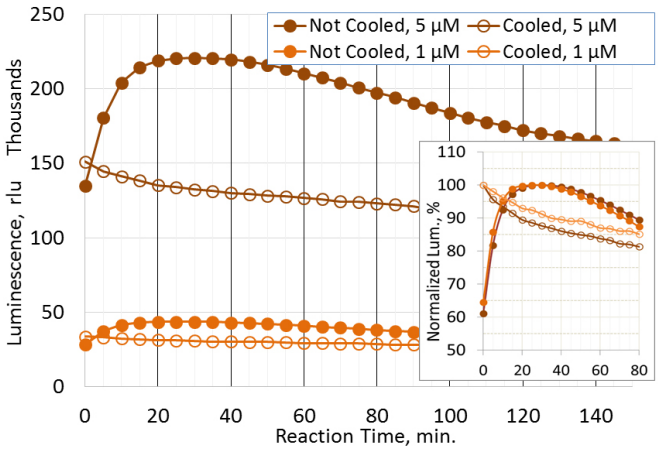

Figure 2: Effect of temperature on the bioluminescent Succinate-Glo assay.

Temperature also affects biofluorescence as well as bioluminescence reaction rates, with fluorescent signals increasing more rapidly without internal cooling than when using the Te-Cool module, as shown by the ApoLive-Glo viability assay results (Figure 3).

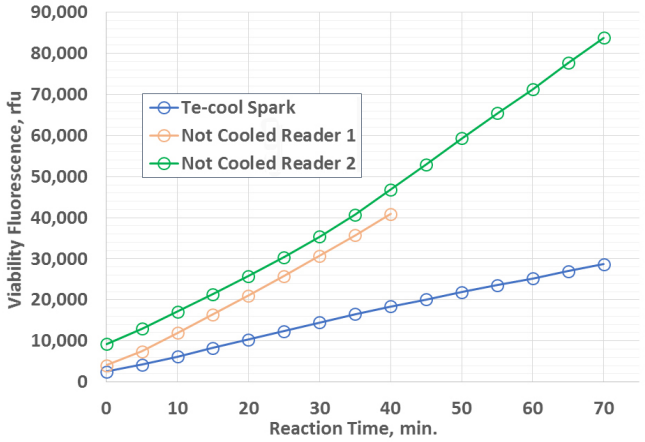

Figure 3: Viability of K562 cells using the ApoLive-Glo assay (Ex = 405 nm; Em = 500 nm).

Different bioluminescent reactions are affected differently by temperature changes

The impact of a reader's internal temperature on the reaction rate is assay dependent. As can be seen in Figure 4, the CellTiter-Glo assay shows a signal intensity change of only 5 %, while the UDP-Glo assay shows a signal intensity change of approximately 20 %.

The ADP-Glo assay shows the largest change, exhibiting an increase by up to 40%.

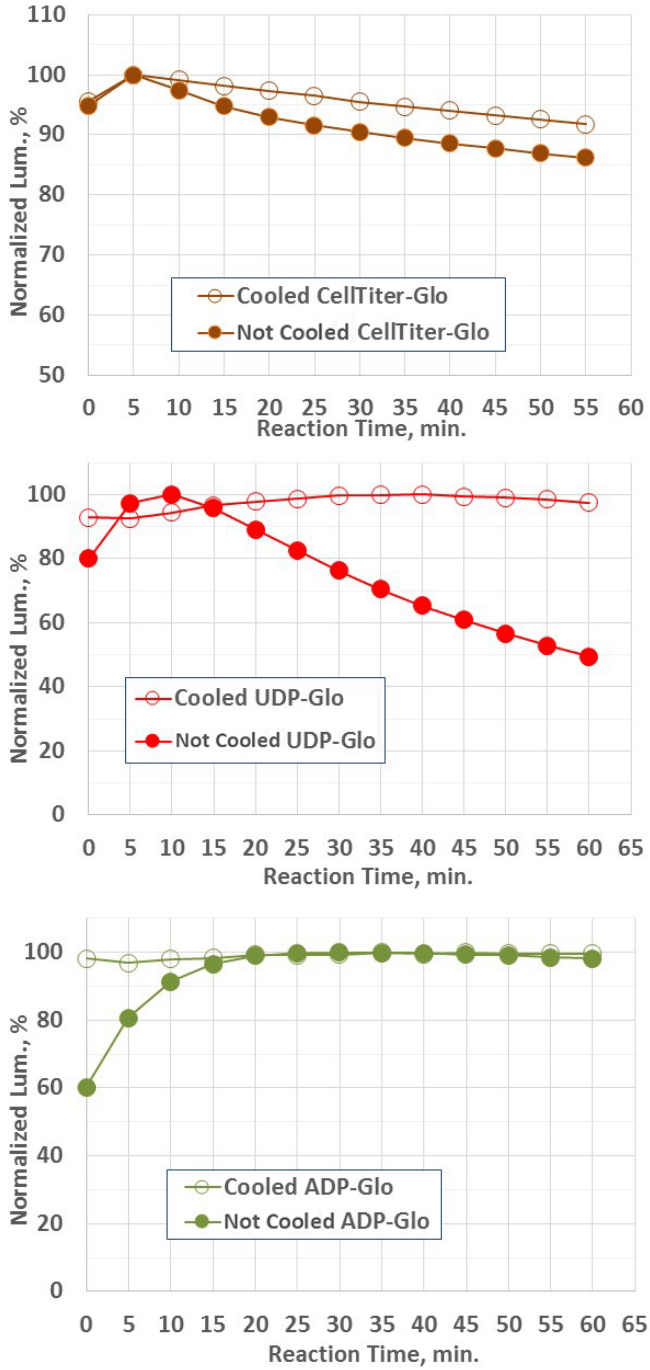

Figure 4: Comparison of results for three different bioluminescent assays in cooled vs. non-cooled readers.

Even small temperature changes affect reaction rates and signal intensities

Small variations in a reader's internal temperature can have a demonstrable effect on the luminescence signal. Figure 5 shows how changing the internal temperature of the Spark reader by as little as 1.5°C using the Te-Cool module causes a marked increase in the luminescent signal. The larger the temperature increase, the larger the signal change.

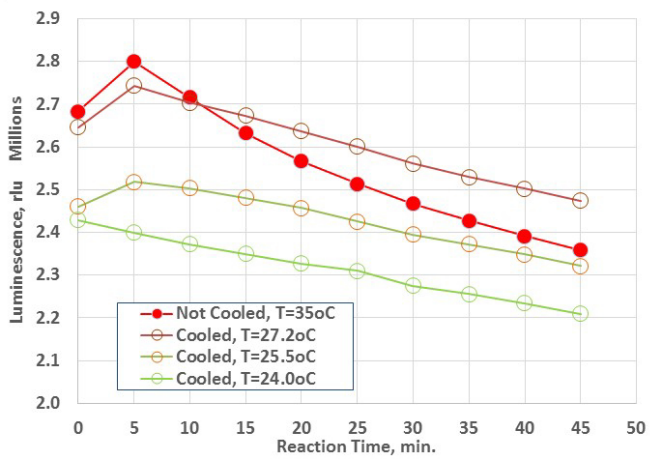

Figure 5: Measured luminescent signal of a Kinase-Glo assay without cooling (at a room temperature of approximately 24 °C) and using the Te-Cool module set at various temperatures.

LOPAC library screening with the Succinate-Glo assay in a cooled vs. non-cooled reader

The luminescence of a set of 384-well LOPAC library plates (16 plates) was measured using the Spark with active cooling and a non-cooled reader. Results obtained with the cooled Spark reader show a narrower signal intensity distribution (Figure 6) than data obtained on the non-cooled reader (Figure 7). Without cooling, temperature increases during the measurement period lead to significantly higher luminescence signals towards the end of each plate read, resulting in a distinctly visible 'jigsaw' data pattern.

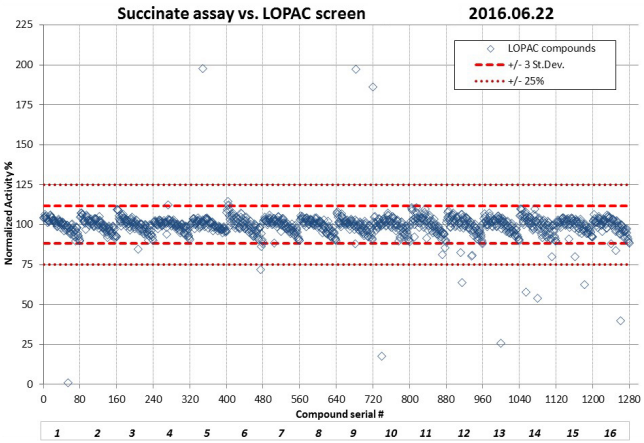

Figure 6: LOPAC library screening on the Spark reader with Te-Cool temperature control. Each 384-well plate contained 80 LOPAC compounds in quadruplicate.

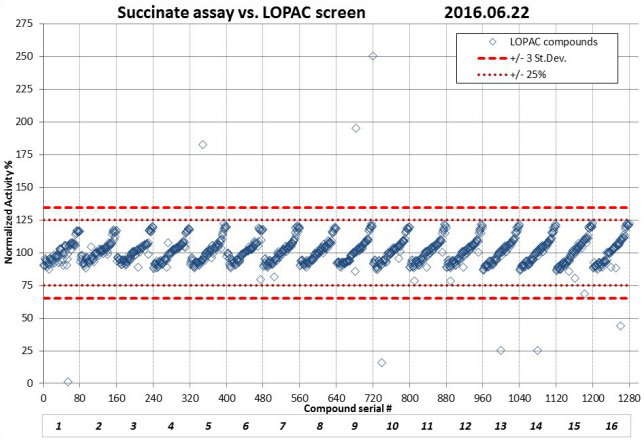

Figure 7: LOPAC library screening on a non-cooled reader. Each 384-well plate contained 80 LOPAC compounds in quadruplicate.

The Z' factor is a statistical function commonly used to judge the robustness of high throughput screening assays [1]. Assays resulting in a Z' factor greater than 0.5 are commonly considered reliable. The Z' factor is calculated using the following formula:

Z' = 1 – ((3*SD of sample + 3*SD of control) / (MEAN of sample – MEAN of control))

Z' values from all 16 LOPAC plates measured with and without cooling are summarized in Figure 8. The average Z' value using the Spark Te-Cool module is 0.79, compared to 0.69 of the non-cooled reader.

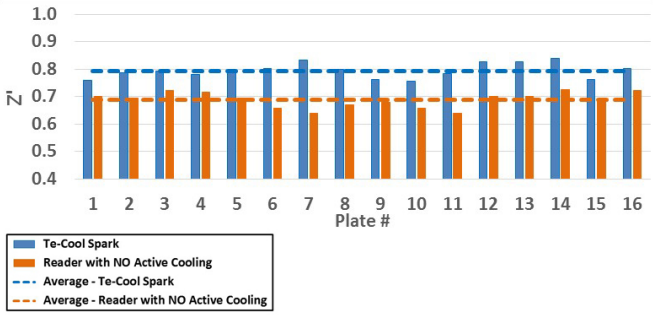

Figure 8: Z' values of measurements taken using the Spark Te-Cool module and a non-cooled reader.

Correlation graphs for LOPAC measurement data obtained using the actively cooled Spark and a non-cooled reader highlight the differences in the luminescence signals caused by the internal temperature of the reader. The significantly higher luminescence signals observed with the non-cooled reader can 'hide' real hits (Figure 9).

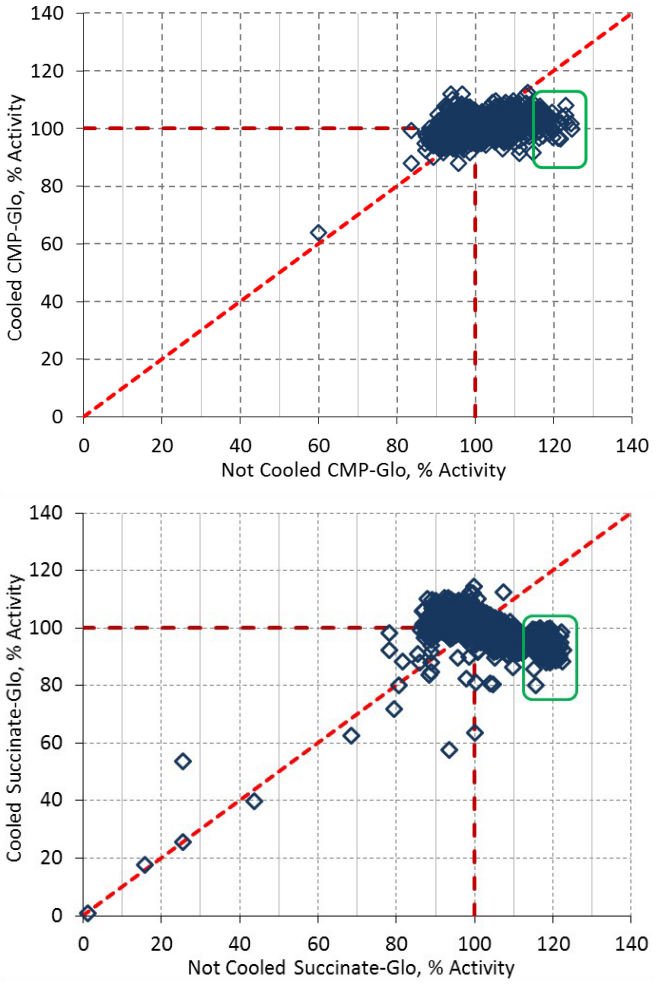

Figure 9: Correlation graphs for CMP-Glo (top) and Succinate-Glo (bottom) assay results obtained using the Spark Te-Cool module and a non-cooled reader. Data points in green rectangles could potentially be masked when a non-cooled reader is used

Pre-warming assay plates before reading can significantly improve data quality. This can be done inside the reader, or in a dedicated incubator set at a temperature equal to the reader's internal temperature. Without active cooling, pre-warming assay plates to 27°C (= the operating temperature of the non-cooled reader) for 10 minutes immediately before reading results in more consistent data for LOPAC screening using the GTPase-Glo assay (Figures 10 and 11).

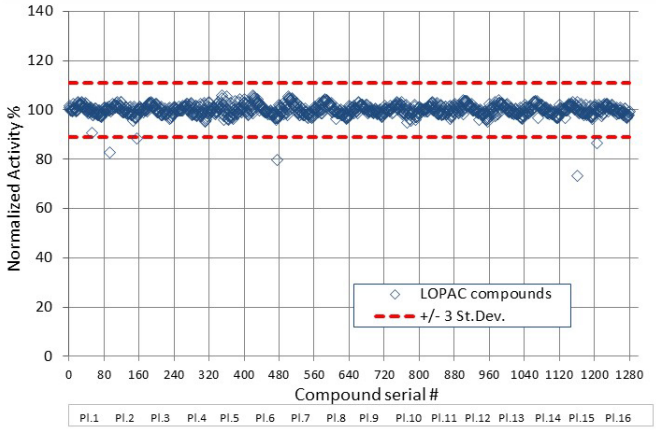

Figure 10: LOPAC library vs. GTPase-Glo assay with pre-warmed plates screen on a non-cooled reader

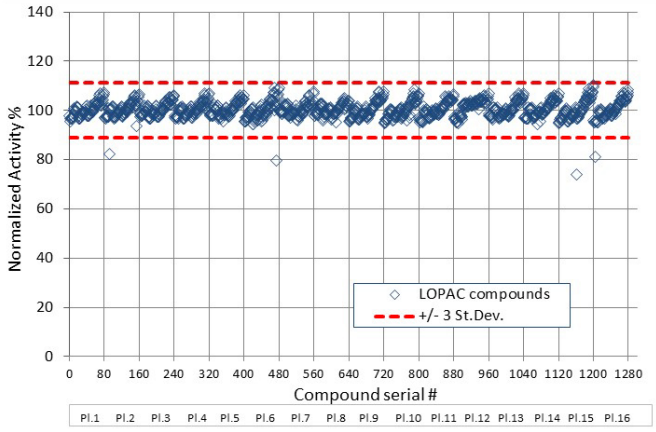

Figure 11: LOPAC library vs. GTPase-Glo assay screen without plate pre-warming on non-cooled reader

Pre-warming the assay plates prior to reading on a non-cooled reader results in an improved Z' value of 0.93 compared to 0.87 without pre-warming, (Figure 12), but extends the overall assay processing time.

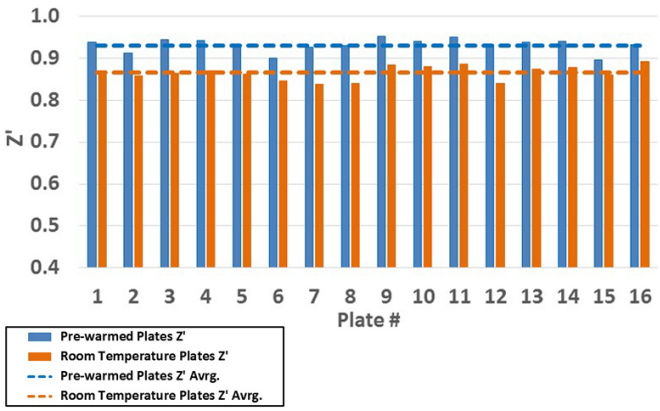

Figure 12: Z' values from non-cooled readers with and without plate pre-warming.

CONCLUSIONS

The internal temperature of a reader can have an observable effect on the rates of both bioluminescent and biofluorescent reactions, and this effect is dependent on the assay. With non-cooled readers, there can be a significant increase in bioluminescent signals and reaction rates during reaction solutions equilibrating to the internal temperature of the reader. This effect can be mitigated by pre-warming the assay plates, but extends experimental times.

The Spark's integrated Te-Cool module enables the temperature inside the reader to be precisely adjusted to match the desired reaction temperature. Maintaining a constant reaction temperature allows true reaction rates and signal intensities to be determined. This improves data quality and provides better assay windows, helping to, for example, identify screening hits that would otherwise be hidden by the dispersed signal.

The results summarized in this application note show that the integrated cooling function of the Spark multimode reader helps to stabilize temperature-dependent assays, making the results more reproducible and reliable.

ABBREVIATIONS

Avrg	Average
CMP	Cytosine monophosphate
GTP	Guanosine triphosphate
LOPAC	Library of pharmacologically-active compounds
UDP	Uridine diphosphate

REFERENCES

1) Zhang JH, Chung TD, Oldenburg KR. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen, 1999, 4, 67-73.

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