

Optimization of temperature-sensitive assays using the Spark[®] Te-Cool[™] •

Application Note

**KEEP CONTROL OF CRUCIAL EXPERIMENTAL CONDITIONS WITH THE FIRST
AIR CONDITIONING SYSTEM FOR A MULTIMODE READER – ENABLING STABLE
MEASUREMENT CHAMBER TEMPERATURES DOWN TO 18 °C.**



INTRODUCTION

Scientific outcomes are influenced by many different factors. It is therefore essential to define and generate stable experimental conditions, including environmental factors such as the temperature, for reliable and reproducible results. It is well known that the rate of a chemical reaction, described by the Van't Hoff equation¹, is highly temperature dependent. However, the temperature in laboratories around the world will differ depending on the local climate, the season and even the time of day (Figure 1).

Multimode readers are popular tools used for both endpoint and kinetic measurements, but the temperature inside the measurement chamber of a reader is not necessarily equal to the room temperature. The electronics inside the instrument can cause the chamber temperature to increase to as much as +4 °C above ambient, especially if the reader has been running for a while.

Until now, it has only been possible to heat multimode readers using temperature control, not to set them at ambient temperature or lower. This can cause problems for temperature-sensitive experiments, such as enzymatic assays, affecting the reproducibility of results over time. In addition, the experimental precision can be decreased by the plate heating up during measurements, even over just a few minutes.

Tecan's Te-Cool module for the Spark platform, the first air conditioning system for a multimode reader, addresses this problem. This unique device can cool the measurement chamber, enabling it to be set at any user-defined temperature from 18 to 42 °C, guaranteeing stable conditions every time, in every lab, across every season, anywhere in the world.

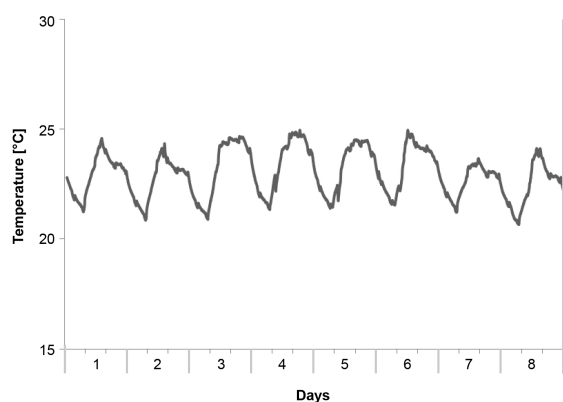


Figure 1: Example of temperature fluctuations in a laboratory. The temperature fluctuates by around 4 °C, and is higher during the day than in the night.

MATERIALS & METHODS

Temperature-dependent signal intensities in an enzymatic assay

A luminescent kinase assay was used to assess enzymatic temperature dependency. Ultra-pure ADP was added to the reaction buffer containing the luciferase. 20 µl of the reaction mix was pipetted into the edge and center wells of a 384-well plate (see Figure 2 for plate layout). The remaining wells were filled with water.



Figure 2: Pipetting scheme for glow luminescence measurements in a 384-well plate. Wells filled with luciferase and reaction buffer are colored yellow, wells filled with water are blue.

The plate was equilibrated to ambient temperature (22 °C) for at least 10 min before measuring the luminescent signal at different temperatures (18, 22 (ambient), 26 and 30 °C) in a Spark multimode reader, performing endpoint and kinetic measurements for up to 10 min (Table 1).

Measurement parameters

Plate type	Greiner® Bio-One 384 Flat White [GRE384sw]
Temperature	18, 22 (ambient), 26 or 30 °C
Measurement mode	Luminescence
Attenuation	Automatic
Settle time	0 msec
Integration time	200 msec
Output	Counts/sec

Table 1: Measurement parameters used for luminescence measurements.

Temperature-dependent enzyme stability

Another critical point in enzymatic assays is enzyme stability. A sequential luciferase assay was performed to demonstrate the decrease in enzyme stability at increasing temperature, measuring purified luciferases from *Renilla reniformis* (sea pansy) and *Photinus pyralis* (firefly beetle). The luciferases were pipetted into the wells of a 96-well plate. The plate was transferred into the Spark reader set to different temperatures (18, 22 (ambient), 26 and 30 °C), and the luminescent signal of each luciferase was detected sequentially for 10 sec directly after injection of 100 µl of the corresponding substrate (Table 2).

Measurement parameters

Plate type	Greiner Bio-One 96 Flat White [GRE96fw_chimney]
Temperature	18, 22 (ambient), 26 or 30 °C
Well-wise measurement	
Measurement mode	Luminescence – well wise

Label 1 – Luciferase 1 (firefly luciferase)

Inject	Injector A; 100 µl substrate 1; 200 µl/sec; refill after injection
Wait	3 sec
Measure	Luminescence
Attenuation	Automatic
Integration time	10,000 msec
Settle time	0 msec
Output	Counts/sec

Label 2 – Luciferase 2 (*Renilla* luciferase)

Inject	Injector B; 100 µl substrate 2; 200 µl/sec; refill after injection
Wait	3 sec
Measure	Luminescence
Attenuation	Automatic
Integration time	10,000 msec
Settle time	0 msec
Output	Counts/sec

Table 2: Measurement parameters for a double luciferase assay consisting of two sequential luminescence measurements.

RESULTS

Temperature-dependent signal intensities in an enzymatic assay

The conversion rate in enzymatic reactions is often temperature dependent, with increased reaction rates at higher temperatures. This behavior was observed in an exemplary luminescent kinase assay, where the activity of a glow luciferase was measured at different temperatures (Figure 3). The luminescent signal increased approximately 10 % per 1 °C increase in temperature.

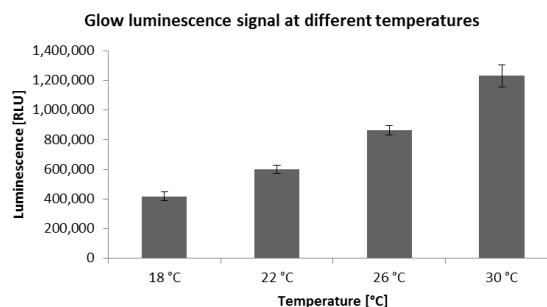


Figure 3: The luminescent signal of an exemplary glow luciferase is temperature dependent. Increasing temperature results in a higher luminescent signal.

During kinetic measurements, the luminescent signal in plates equilibrated to room temperature was constant when the Spark reader was also equilibrated to room temperature (Figure 4A). Increasing the temperature inside the reader resulted in a higher luminescent signal compared to the initial values, due to the plate heating up. Over a measurement time of just 6 min – a duration which can be reached for measurements of a complete 384-well plate – the luminescent signal was increased by 34 % at 4 °C above ambient and 78 % at 8 °C above ambient, compared to a 2 % reduction in signal intensity when operating the Spark reader at ambient temperature. Additionally, the coefficient of variation (CV) of the results significantly increased at higher measurement temperatures (Figure 4B).



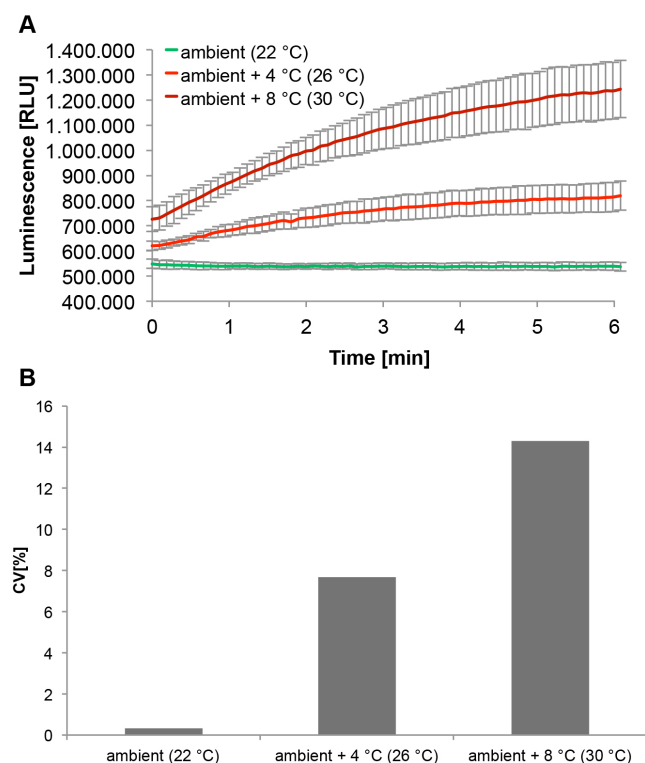


Figure 4: Luminescent signal over time in a kinetic measurement. During a measurement time of 6 min, the luminescent signal increases due to heating of the liquid in the wells (A). This results in elevated signal variations and higher CVs for the measurement results (B).

Temperature-dependent enzyme stability

Assay temperature does not necessarily affect enzyme activity. Some enzymes might show altered conversion rates at different temperatures, while others show decreased stability. This was demonstrated in a luminescence assay where firefly and *Renilla* luciferases were detected sequentially during a well-wise measurement at different reader temperatures. The protocol for this assay required relatively long integration times, resulting in a measurement time of almost 30 sec per well, and therefore taking around 45 min for a complete 96-well plate. Figure 5 shows that the luminescent signal for the firefly luciferase decreased over time as a consequence of protein instability at elevated temperature, especially at 30 °C (A). In contrast, *Renilla* luciferase was not affected by altered temperatures inside the Spark reader (B). The effect of temperature on enzyme activity becomes obvious when the CV over multiple plate wells is compared (Figure 6).

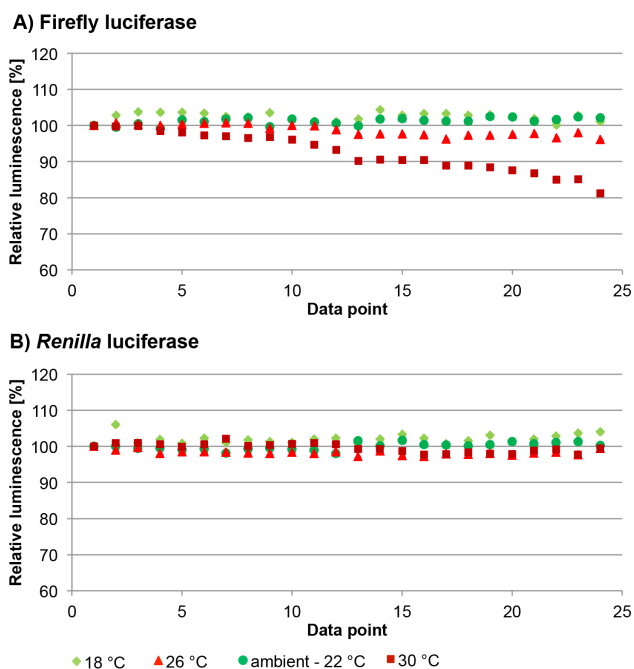


Figure 5: Sequential luminescence assay employing firefly luciferase and *Renilla* luciferase. While the signal of *Renilla* luciferase is stable with increasing temperature (B), elevated temperature decreases the activity of firefly luciferase (A).

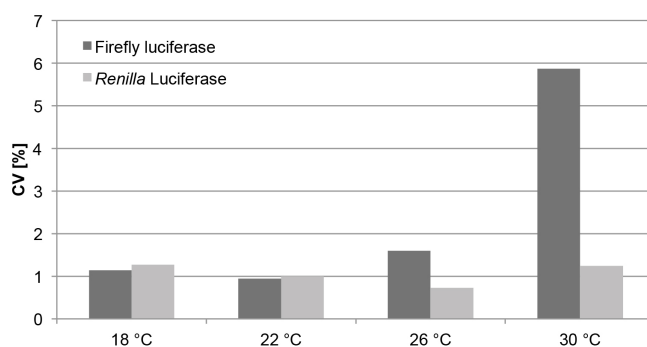


Figure 6: Temperature-dependent decrease in firefly luciferase activity is shown by the higher CV at 30 °C.

CONCLUSION

The data presented in this application note confirms that temperature is an important parameter for chemical reactions in general, and enzymatic assays in particular. Here, three different enzymes were used as examples of the potential temperature dependency of enzyme activity. Two of the three enzymes were strongly affected by temperature change, resulting in altered activity or reduced stability. For optimal data quality it is therefore essential to control this parameter during an experiment.

The Te-Cool temperature control module for the Spark multimode reader allows not only heating of the reader but also cooling, even below ambient temperature. This enables conditions to be precisely defined to meet the requirements of the assay, improving the quality of experiments by ensuring enhanced reproducibility and precision for each measurement. This unique module therefore guarantees optimized results, every time, in every lab, across every season, anywhere in the world.

ABBREVIATIONS

CV Coefficient of variation

REFERENCES

- 1) Atkins, Peter; De Paula, Julio (10 March 2006). Physical Chemistry (8th ed.). W.H. Freeman and Company. p. 212. ISBN 0-7167-8759-8

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