1257-C

Improving your data with Internally Cooled Multifunctional Reader

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1. Introduction

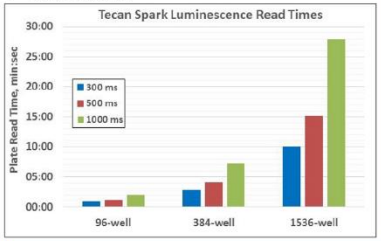
Temperature is considered a major factor that affects the rate of chemical reactions.
The average speed of a reaction is related to the square root of the absolute temperature.
Arrhenius' equation describes this dependence: $k = Ae^{-E_a/(RT)}$
For most biological systems, the rate of change as a consequence of increasing the temperature by 10°C (Q_{10} value) is ~ 2 to 3.

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. The Tecan Spark® plate reader with Te-Cool™ module allows adjustment of the instrument's internal working temperature from 18°C to 42°C.
Having the ability to investigate samples of interest at ambient temperature, can limit heating effects on reaction dynamics. In this work we present the benefits of an actively cooled multimode reader in investigations employing bioluminescence and biofluorescence reactions, kinetic profiling and high throughput screening.
The kinetic profile of enzymatic reactions can appear very different when acquired on readers that are equipped with an internal cooling capability versus instruments that do not have this functionality. This effect can be mitigated by pre-warming assay plates before reading.

2. Luminescence Read Times per Plate and Consequential Plate's Warming up can be Significant

Luminescence read Time per plate depends on:

- ✓ # of wells / plate
- ✓ Read Time / well



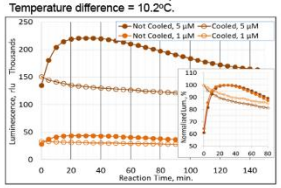
Internal Temperature of Multifunctional readers that are not actively cooled is 2 - 10°C warmer than ambient laboratory temperature.

So, plate inside the reader warms up by up to 10°C causing acceleration of the chemical processes.

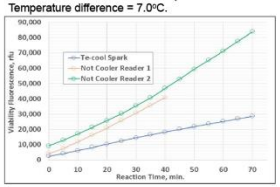
In the work presented 384 well plates were read for 300ms/well, so all wells were read just in under 3min.

3. Both Bioluminescent and Biofluorescent Reaction Rates are Affected by Temperature

Succinate-Glo™ assay at 5µM or 1µM of Succinate in Actively Cooled vs. Non-Cooled Readers.
Room Temperature = 22.6°C;
Non-Cooled reader internal Temperature = 32.8°C;
Temperature difference = 10.2°C.



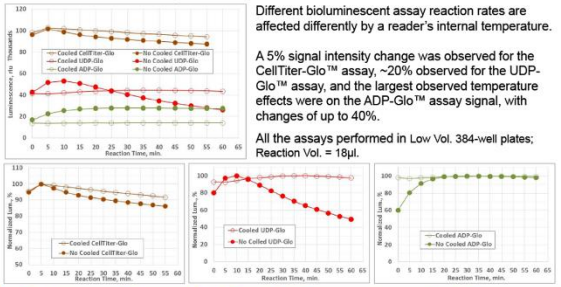
ApoLive-Glo™ assay, Fluorescence Viability signal of K562 cells in Actively Cooled vs. Non-Cooled Readers.
Room Temperature = 21.0°C;
Non-Cooled Reader 1 inside Temperature = 28.0°C;
Temperature difference = 7.0°C.



- The internal temperature of a reader affects both bioluminescent and biofluorescent reaction rates.
- Reaction rate is significantly faster at elevated temperatures inside a non-cooled reader.
- Temperature effect on bioluminescence reaction rate is concentration independent.

4. Different Bioluminescent Reactions are Affected Differently by Reader Internal Temperature

Three bioluminescence assays in Cooled vs. Non-Cooled readers



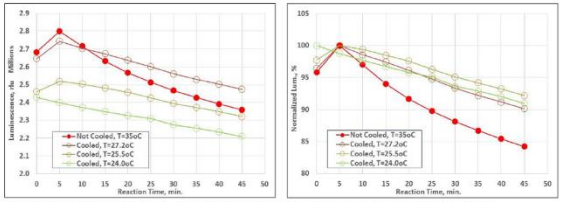
Different bioluminescent assay reaction rates are affected differently by a reader's internal temperature.

A 5% signal intensity change was observed for the CellTiter-Glo™ assay, ~20% observed for the UDP-Glo™ assay, and the largest observed temperature effects were on the ADP-Glo™ assay signal, with changes of up to 40%.

All the assays performed in Low Vol. 384-well plates; Reaction Vol. = 18µl.

5. Internal Te-cool™ Spark® Temperature Can Be Fine-Tuned to Match Room Temperature

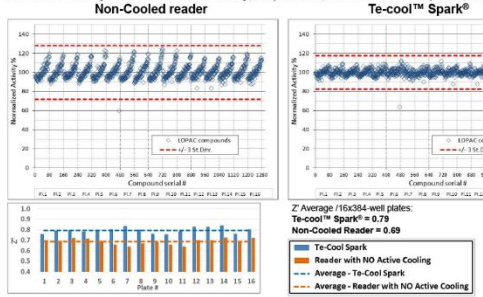
Kinase-Glo™ Assay Luminescence signal in Non-Cooled and Te-cool™ Spark® readers; Low Volume 384-well plates; Reaction Volume = 18µl; Room temperature = 24 °C.



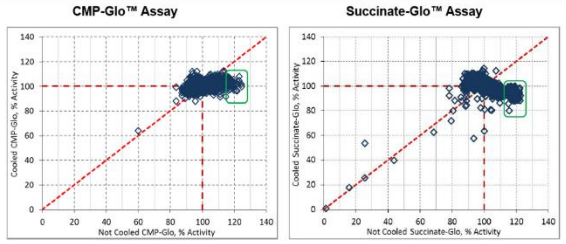
Internal Te-cool™ Spark® temperature can be adjusted to precisely match room temperature. When reaction temperature remains steady, bioluminescent signal shows a steady decrease. In Non-Cooled readers we usually observed a bioluminescence reaction signal increase during the first few reads. This is attributed to the reaction solution equilibration to the internal temperature of the reader and concurrent reaction rate increase.

6. LOPAC Library Screens: Te-cool™ Spark® and Non Actively Cooled Reader

CMP-Glo™ Assay screen vs. LOPAC library; 0.5µM CMP; Low Vol. 384-well plates; Total Vol. =10µl.



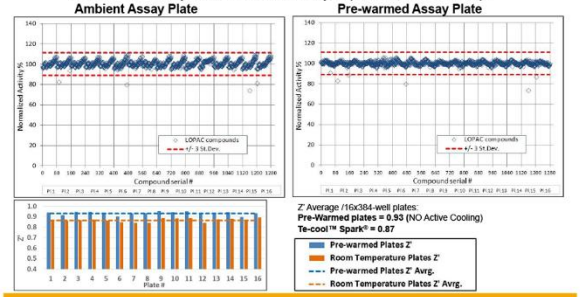
7. LOPAC Screening Results Correlation Graphs: Te-cool™ Spark® vs. Non-Cooled Reader



Temperature equilibration within a Non-Cooled reader leads to greater assay variability and can obscure "Hits"

8. Plate Pre-warming Mitigates the Problem on Non-Cooled Readers

GTPase-Glo™ Assay screen vs. LOPAC library; 10µM GTP; Total Vol. =18µl



9. Conclusions

Temperature effects on chemical reactions

- Chemical reaction rates are significantly impacted by a reader's internal temperature.
- Both bioluminescence and biofluorescence reaction rates are affected by a reader's internal temperature.
- The extent of the temperature effect can vary based on the specific bioluminescent assay.
- When reaction temperature remains constant, bioluminescent reaction rate remains steady and luminescence signal decreases steadily.
- Bioluminescent assay-based small compound library screen results can be distorted due to the plate reader's internal heat effect on bioluminescent assay.
- Pre-warming assay plates prior to a read can successfully mitigate the effects of a reader's internal temperature.

Benefits of Actively Cooled reader

- With the internal Te-cool™ module the temperature inside the Spark® reader can be adjusted precisely to match the reaction temperature of the plate.
- Constant reaction temperature outside and inside the reader:
  - Allows detection of the true reaction rate and signal intensity.
  - Tightens assay windows to expose more "Hits".

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