

# Agilent BioTek Gen5 Discontinuous Kinetics for Long-Term Kinetics

Monitoring algal cell growth for biofuel applications

## Introduction

Long-term growth measurements usually require cultures to be maintained under optimal growth conditions, with an aliquot tested periodically, as the microplate reader may not be ideal for optimal growth. For example, algal cultures often require polychromatic light for photosynthetic growth, while the read chamber of a microplate reader is normally dark and light-tight by design. Also, cell cultures demand humidity levels approaching 100% to prevent evaporative loss. In addition, long-term growth monitoring often only requires one or two measurements per day for weeks. Dedicating a microplate reader for so long, at the exclusion of all other experiments, is not practical. Providing a second dedicated reader is cost-prohibitive. The traditional solution has been to read the samples as a traditional endpoint read and collate the data manually at the end of the experiment. Using the discontinuous kinetics option in Agilent BioTek Gen5 data analysis software offers a solution to this problem.

Discontinuous kinetics provide a means for a long-term kinetic reading with multiple measurements on a plate, or a series of plates, over any period without tying up the reader. This option allows removal of the plate for processing after each read interval, so other experiments can be conducted with the reader in between read intervals. When enabled, the discontinuous kinetic option uses the internal clock of the PC to provide a time and date stamp to each read event, allowing Gen5 to keep track of the total run time of the entire assay.

## Selecting discontinuous kinetics

To use the discontinuous kinetics option, a New Experiment file needs to be created that either enables the feature directly, or uses a pre-existing Gen5 Protocol file with the feature enabled. It is critical that this option is enabled before running the assay. Discontinuous kinetics is enabled from the Advanced Options button or Options link located in the Protocol Procedure window.

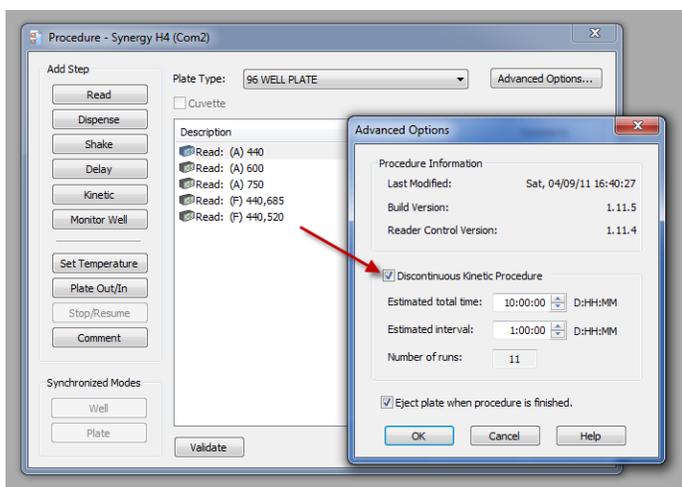


Figure 1. Advanced Options window.

In the Advanced Options window is a check box to enable discontinuous kinetics (Figure 1, red arrow). Once Discontinuous Kinetics has been enabled, Gen5 requires that an "estimated" number total run time Days:Hours:Minutes as well as an expected read interval be entered. These are just an estimate and will not interfere with actual experiment activity. Gen5 uses your estimated time lines to set up the data views options and formulas with placeholders until the actual data are captured.

When in doubt about the required time period and intervals, it is best to overestimate them so that the number of read values is maximized. In Figure 1, the "Eject plate when procedure is finished" option is enabled. This option (when supported by the reader) gives you more control over plate processing. The default setting mirrors most Agilent BioTek readers: it ejects the plate when the Read/Procedure is completed. If a situation demands that the plate remain inside the reader upon the completion of the read, one can deselect the option to keep the plate inside the reader at the end of a Procedure.

## Example

*Chlorella vulgaris* algae growth can be used as an example to demonstrate the discontinuous kinetics procedure. As demonstrated in Figure 2, the growth of this single cell algal strain was monitored over a period of 17 days using light scattering absorbance as an indicator of cell growth. Over time, the measured absorbance at 600 nm increases indicating an increase in cell number. Note that while a single measurement was taken daily, the data points are not at evenly spaced intervals, indicating that plate measurements were not necessarily at the same time each day or that a measurement point was missed.

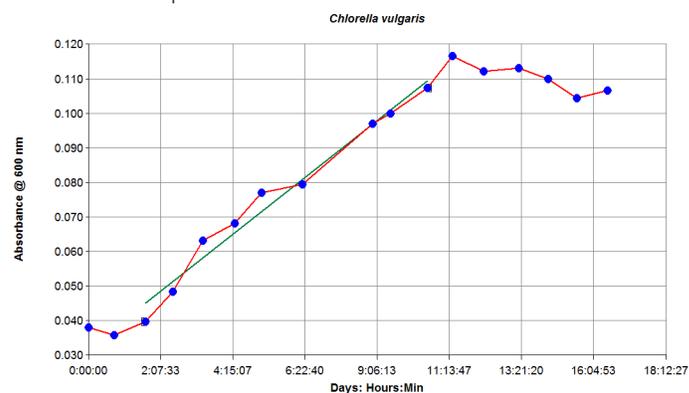


Figure 2. Agilent BioTek Gen5 kinetic plot of *Chlorella vulgaris* algae growth. The Agilent BioTek Gen5 discontinuous kinetics procedure was used to maintain a data timeline. A mean slope value (green line) was determined from data points 3 to 11.

## Results and discussion

The purpose of discontinuous kinetics is to link a series of endpoint measurements together regarding time in a manner similar to traditional kinetic measurements, and provide the same data reduction tools. Discontinuous kinetics has several features that are distinctly different from traditional kinetics. As previously stated, discontinuous kinetics allows the user to make kinetic measurements over a long period without tying up the reader during the intervals between reads. In addition, it also allows flexibility in the read interval. Because the plate read is initiated manually, the time between reads is not rigidly fixed. Data are plotted based on the total run time at the time of the plate read. All kinetic calculations are plotted as a function of the recorded time. This allows for calculations, such as mean slope  $V$  or maximum slope  $V$ , to be automatically determined by Agilent BioTek Gen5 data analysis software regardless of the actual measurement interval. The discontinuous kinetics procedure can also be used to monitor several plates over time. This is advantageous when an Agilent BioTek reader is used in conjunction with an Agilent BioTek BioStack microplate stacker. Several plates can be read in sequence for any number of cycles without any manual intervention, with kinetic curves being generated for each well of each plate automatically by the Gen5 data analysis software.

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