Science Education Collection

# Introduction to the Microplate Reader

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## Abstract

The microplate reader is a multimodal instrument that allows for a variety of experiments to be performed and measured simultaneously. Microplate readers can make absorbance, fluorescence and luminescence measurements. Multiwell plates are integral to the microplate reader and allow for many experiments to be performed at once. Regardless of the assay type, experiments on the plate reader utilize a standard curve to determine the experimental values. This curve uses samples of known concentration to generate a line of best fit or standard curve. Experimental values are then extrapolated to the curve or are calculated using the equation from the linear regression. Besides standards and samples being run on the multiwall plate, the blank along with positive and negative controls are also used in the assay to ensure it is working correctly. Multiplate readers are used to quantify protein, gene expression and various metabolic processes such as reactive oxygen species and calcium flux.

## Transcript

The microplate reader is a widely-used instrument that allows for many samples to be simultaneously measured, as if many miniscule experiments were being performed at the same time.

This apparatus is used in conjunction with multiwell plates, like the 96 well plate.

Regardless of the type of experiment run with the microplate reader, standard curves are often used to determine the value of experimental samples, as well as positive and negative controls.

Microplate readers come in different shapes, sizes and set-ups. Many microplate readers have multimodal capabilities allowing for many different assays to be performed. These modalities include the ability to perform different types of measurements, such as absorption, fluorescent, and luminescent measurements.

Multiwell plates are integral components to the microplate reader and are used to hold the samples that are measured by the machine. These plates can be different sizes, have different types of well bottoms and different numbers of wells. The type of plate used depends upon the assay.

The loading tray is used to bring the 96-well plate into the machine.

A computer interface is typically used to operate the plate reader and control its settings and parameters, such as the wavelength and mode. The plate reader software has a graphical user interface of the plate that allows you to select which wells are loaded with samples.

Multichannel pipettes are often used to load multi-well plates. The reservoirs hold the solutions for the multichannel pipette.

Wells can sometimes be loaded using a standard single channel pipette.

Samples and standards are loaded either in duplicate or triplicate to account for any pipetting errors. Here you see a plate loaded in triplicate.

The standard curve uses samples with known concentrations, which yield different absorbance values. This data is then used to create a graph where a line of best fit is generated.

The blank is used to determine the extent of your measurement that is not experimentally relevant and is due to the buffers in which your sample is diluted or reagents to which your sample is exposed. The values obtained from these measurements are called the “background”. The blank does not contain any sample.

The positive control indicates whether or not the assay has worked properly. It gives a good result. The negative control is a control variable where no measurement/effect is expected to be observed. It should not yield any result..

Once the plate is set up, it’s time to load the samples. To prevent measuring the wrong samples or loading the plate the wrong way, it is critical to orient the plate correctly in the loading tray. Remember to exercise caution when loading samples in the tray, so as not to force the tray into the instrument or catch ones extremities inside the instrument.

Once the tray is loaded, parameters such as the mode, wavelength and well loading order are set in the software before the plate is read.

After the parameters are set, the plate is read, and the reader generates a read-out of values within the software.

Once the plate is read, use the average value of the blank samples to subtract the background from all samples including the standard curve.

After reading, the known concentration values for the standards are plotted against their respective measured values, absorbance in this case.

When the values have been plotted, the line of best fit can be calculated using a linear regression. This can be easily done using a spreadsheet program.

The coefficient of determination, a statistical measure of how well the line predicts actual data points, should be between 0.90-0.99, with 0.99 being considered the best value and signifies that the line fits the data perfectly.

Using the line of best fit, we can calculate the concentration values of experimental samples or controls in each well by plugging in the absorbance value for Y and then solving the equation for X. Concentration values can also be estimated by drawing a line from the absorbance value on the Y axis to the best fit line and then down to the X axis.

Many types of microplate readers measure absorbance, which is defined as the logarithmic ratio of light falling upon an object to the light transmitted through an object.

The Bradford assay is an example of an absorbance-based microplate reader assay, where protein samples are added to the plate with the “Bradford” reagent. This compound binds to the proteins in the sample, and cause a shift in its absorbance.

In fluorescent-based assays, a fluorochrome is activated by a certain wavelength of light and in turn causes excitation of the fluorochrome, which emits light at a different wavelength.

When working with light sensitive reagents, be sure to keep them covered to prevent photobleaching and ruining the experiment.

Luminescent assays emit light via a chemical reaction and often use luciferase. Luciferase comes from a number of sources such as fireflies. In a luciferase reaction, light is emitted when luciferase encounters oxygen, ATP, and magnesium in a series of reactions.

Luminescent assays have many different applications. An example of this application is measuring the production and detection of reactive oxygen species in cancers.

Other applications, which use microplate readers, include high-throughput assays using 384- and 1536-wells. In these assays, plates are loaded by a robot. No, not that kind of robot. A programmable robot which automates extremely precise sample handling.

You’ve just watched JoVE’s introduction to the microplate reader. In this video, we showed what a microlate reader is(A), how it is used(B), how to operate this instrument(C), how to interpret microplate reader data, and some applications using a microplate reader(D). Thank you for watching.