

Optimizing the EnVision plate reader for your assay

Optimization calibrates the plate reader to specific parameters of your assay's signal and plate type. It will ensure accuracy of the plate reader for your specific assay. Optimization should be performed with control samples before you read any experimental samples. It may be necessary to optimize your readout more than once, perhaps even each time you use the reader.

Optimization parameters for different readouts

For absorbance, the required optimizations are plate dimension and measurement height. For fluorescence or fluorescence polarization, optimizations for plate dimension, measurement height, and detector gain is necessary. For luminescence, the required optimizations are plate dimension, measurement height, and cross talk.

Table 1. Optimization Parameters

Parameter	Description	Required for absorbance?	Required for fluorescence/FP?	Required for luminescence?
Plate Dimension	Determines the exact size of the plate type and identifies well centers.	✓	✓	✓
Measurement height	Determines the optimal focal point. The plate is measured over a range of vertical positions to determine which provides the best readout.	✓	✓	✓
Detector gain	Determines the optimal gain for the detector. The optimal gain is the factor that results in the maximum possible signal without saturating the detector by high-signal samples.		✓	
Crosstalk correction	Corrects glow to prevent signal crosstalk between adjacent wells.			✓

Performing Optimization

The EnVision software package provides an "optimization wizard" that automatically sets readout parameters based on your control samples (see below). For 384-well and higher density microplates, the plate dimension wizard should always be used. You may wish to set other parameters independently, such as height and gain. This is because the wizard overrides all user-adjustable settings for height and gain. If you use the optimization wizard for height or gain, and then want to adjust those settings, you must delete the previous optimization. The screening room staff will help you decide whether you should use the wizard for specific parameters.

Please note that a protocol is only optimized for the specific EnVision instrument on which the optimization was performed. There are currently 3 EnVision readers at the ICCB-L/NSRB facility. If you wish to use the same protocol on a different EnVision, you must transfer the protocol to the new plate reader and re-optimize on the new reader.

Control Samples for Optimization

You will need to prepare control samples for your optimization plate. You should use the same reagent concentrations and volumes that you will use for experimental assays.

Figures 1 and 2 show examples of plate layouts for optimization. The screening facility staff may recommend alternative layouts depending on your situation. The term "maximal signal" refers to a sample that provides the highest signal that is expected under experimental conditions. For example, if you want to monitor a decrease in signal from a baseline level, you should use your baseline condition as the maximal signal sample. If you want to monitor an increase in signal, you should use the condition that corresponds to the maximal expected signal. In Figure 2, the term "background signal" refers to an appropriate control sample that is not induced for luminescent signal (e.g., no luciferase reagent/substrate added).

Figure 1. Sample placement for optimization of absorbance, fluorescence, or fluorescence polarization readouts in 384-well plates.



