

Dual Wavelength Analysis: Are 2 wavelengths required?

The protocols in our ELISA kits recommend the use of dual wavelength analysis for microtiter plates readers appropriately equipped. For alkaline phosphatase – PNPP based assays the two wavelengths are 405nm and 492nm. For horse radish peroxidase – TMB based assays the wavelengths are 450nm and 630nm. In both assay types the first wavelength listed, also termed the “test wavelength”, is critical and thus your microtiter plate reader should be equipped with the appropriate 405 and 450 nm filters. It is not absolutely necessary to use dual wavelength analysis with any of our assays and thus the second wavelength (492nm and 630nm - also called the “reference wavelengths”) are optional. In theory, the use of dual wavelength analysis should provide better precision. Microtiter plate readers with the appropriate software will automatically subtract the reference wavelength absorbance from the test wavelength absorbance. In this way if there were any non-wavelength specific imperfections in the optical system, dual wavelength analysis could help to overcome them. In practice because the optical quality of our microtiter plates is excellent, there is usually no significant statistical improvement in the data by subtracting out the reference absorbance from the test absorbance. Most plate readers are equipped to perform dual wavelength analysis, however, in this case it is not a very significant improvement. Since the reference wavelength is not critical, microtiter plate readers with reference filters close to those we recommend can be substituted. For example you may use a 490nm or a 500nm filter in place of the 492nm or a 600nm or 650nm filter in place of the 630 nm filter.