

## Procedure for Determination of Percentage Difference in Reduction between treated and control cells in Cytotoxicity / Proliferation Assays

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- A correct factor (**RO**) for the absorbance of oxidized **KineticBlue** must be calculated.
- Measure the absorbance (AM) of growth medium alone. (without addition of **KineticBlue**)
- Measure the absorbance of oxidized (blue) **KineticBlue** in growth medium at the low and high wavelengths.
- Subtract (AM) from each of the measured **KineticBlue** absorbance to produce, respectively, AOLW and AOHW. These are absorbance of oxidized (blue) **KineticBlue** at the low and high wavelengths respectively.
- Calculate the correction factor RO of oxidized **KineticBlue** :

$$(RO) = (AOLW) / (AOHW)$$

- Measure the absorbance values (ALW and AHW) of a test sample at each wavelength.
- Calculate the percentage of reduced **KineticBlue** (ARLW) in a sample as :

$$ARLW = 100 \times [ALW - (AHW \times RO)]$$

- Calculate the percentage difference in reduction (PDR) between treated & control cells :

$$PDR = 100 \times (\text{test ARLW} / \text{positive growth control ARLW})$$