



Fig. 1 (a) Variation of key parameters and conditions in order to establish the linear range of a cell-based assay. Some definitions: the linear range comprises the optimal region in which the actual assay read-out is proportional to a particular cell number (i.e. targets/cell) and the parameters/conditions varied, respectively. In contrast to the linear range, the dynamic range of an assay is defined as the area between the mean maximal and mean minimal signals (*background*). The crucial working range is obtained by subtracting 3 standard deviations (SDs; σ) from the maximal signal and adding 3 SDs to the minimal signal/background. Any value within the working range can be considered statistically significantly different with 99.7% confidence. **(b)** In cases in which **high and low controls are available only**, the calculation of the actual size of the assay window follows exactly the same procedure used to determine the working range of a given assay. In both cases it is obvious immediately that the degree of variation in the highest and lowest signals determines the actual assay quality: even assays with quite low S/B values can be evaluated with high precision once their maximal and minimal values do not oscillate very much – something generally achieved in a straightforward manner when switching from manual well-to-well pipetting to highly accurate electronic multichannel pipettes and dispensers

become a highly rewarding and mandatory routine to corroborate the results obtained.

Reporter gene assays rank first when it comes to studying and monitoring the seemingly innumerable number of cellular processes and signalling pathways in a cost-effective manner, although at the expense of having to deal with a time-delayed read-out due to the transcriptional and translational processes involved. The cell type of interest is either transiently or stably transfected to allow for the expression of an easily and sensitively detected reporter protein in response to a certain stimulus or process.