Quantitative Assay Evaluation and Optimization

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The Z´-factor[1] calculation is useful during piloting for quality assessment of assay conditions. An assay can be considered validated for high-throughput screening after 3 independent experiments (for example, each experiment set up separately starting from scratch or carried out on different days) have been shown to result in reproducible and suitable Z´-factor values. Each experiment should be performed on at least 1 full 384 well plate where ½ of the wells contain positive controls and ½ of the wells contain negative controls. This will produce a statistically significant data set for evaluation.

To quantitatively rank assay conditions, perform control experiments and calculate Z´ from the data collected.

$$Z' = 1 - \frac{(3SD_{+} + 3SD_{-})}{|\, Ave_{+} - Ave_{-} \,|}$$

SD_{+} = positive control standard deviation
SD_{-} = negative control standard deviation
Ave_{+} = positive control average
Ave_{-} = negative control average

Note – Microsoft Excel has a STDEV function that works well for this calculation

After calculating Z´ the following table is useful for evaluating the potential performance you might expect using this assay to screen at the ICCB-L screening facility. If optimization is needed, different assay conditions should be compared and ranked by their Z´-factor values until suitable conditions are found.

High-throughput Screening Assay Fitness Table

1 > Z´ > 0.9	An excellent assay
0.9 > Z´ > 0.7	A good assay
0.7 > Z´ > 0.5	Hit selection will benefit significantly from any improvement
0.5 = Z´	The absolute minimum recommend for high throughput screening

This table may differ slightly from published recommendations. However it is based on the general experience of researchers in the ICCB-Longwood Screening Facility. We commonly observe that screening results rarely achieve the high quality levels seen during piloting using controls.

Please contact us if you require more information.

[1]Ji-Hu Zhang, Thomas D. Y. Chung and Kevin R. Oldenburg (1999). A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen* 4:67-73.