***Instructional Text for Running the Excel kinact/KI Calculator***

**Generalities**

Only enter information in the orange 'input' cells.

After fitting, Save As under a new filename.

**Specifics**

On the Data sheet, enter the name of inhibitor and date of evaluation.

Then enter the parameters for the enzyme reaction of the activity assay.

This information will not change from run to run.

This includes the enzyme concentration, the substrate concentration,

the substrate KM value and the reaction kcat value,

the total volume of the assay mix during this pre-incubation,

and the total volume of the assay mix during the incubation step

(representing a slight dilution of the pre-incubation mixture).

Then enter the raw data for each IC50 curve.

This data set includes the pre-incubation time

(where enzyme is incubated with inhibitor prior to addition of substrate),

the incubation time

(which begins with addition of substrate and ends with reaction quenching/reading)

and finally the normalised % signal measured at each inhibitor concentration.

Next, enter an estimate 'Response coefficient' value for each data set.

This is a scalar value that mirrors the normalisation process.

Enter a value that brings the 'Predicted signal' up to ~100% in the presence of 'zero' inhibitor.

For experiments in which the incubation time does not change from curve to curve, use the same number for each data set.

Next, enter initial estimate values for kinact and KI.

The lines of the predicted values appearing in the curves shown in column I will reflect the initial values, allowing for some manual adjustment.

The KI value will shift the predicted curve along the x-axis, while the kinact value will alter the degree of time-dependence.

Otherwise, values of 1 min-1 and 1 µM are reasonable initial estimates.

Finally, navigate to the 'Data' menu and click on 'Solver'. The conditions should be pre-set, so you can just click 'Solve'.

Solver will optimise the values of cells G1 and G2, in order to minimise the RSS shown in cell G11.

After a few iterations, the best fit values for kinact and KI will be reported in the same cells where you entered the initial estimates.

The goodness of fit can be interpreted from the correlation (R2) and from the RMS error (the typical vertical distance from the best fit line to a data point).

On the 'Report' sheet you will find a printable format of the results,

including the final values for the kinetic parameters,

the goodness of fit measurements, and the IC50 datasets including the predicted values.