

Method Procedure

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Title:	Calculating IC50 values using GraphPad Prism 6 software
Version:	v4
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SOP History		
Number	Date	Reason for Change
v1	11/07/2014	Original
v2	10/07/2016	Update
v3	10/07/2018	Update
v4	10/07/2020	Update



This SOP describes the current procedure for Calculating IC50 values using GraphPad Prism 6 software in Laboratory 248 at the St Andrews School of Medicine (SASoM).

# 2.0 Scope -

This SOP applies to all staff in the SASoM calculating IC50 values using GraphPad Prism 6 software.

## 3.0 Responsibilities -

All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.



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### 4.0 Procedure –

- 1. Go to http://graphpad.com/guides/prism/6/curve-fitting/ for more information.
- 2. Open GraphPad Prism software.
- 3. Create an XY data table.
- 4. Select 'X'
- 5. Select 'Y' 'Enter and plot Single Y value'
- 6. X-values: Enter the concentration of the inhibitor into column X. \*Note: For the control (no inhibitor), simply enter a low dose perhaps 10-10 (0.000000001). You cannot enter zero because zero cannot be defined on a log scale (see later).
- 7. Enter response into Y. Enter one data set into column A and use columns B, C and D etc. for different data sets, if required.
- 8. Since all dose-response equations in Prism expect the X values to be the logarithm of the dose, the data must be 'log transformed'. Select / highlight the complete list of 'X' values. Select Transform. Select 'Transform X values using X=log(X).
- 9. Select 'Fit curve with non-linear regression'. Select 'Dose response inhibition'. Select 'log inhibition with response 3 parameter'. Select OK.
- 10. To normalize the response from 0 to 100% (if not already done), click 'Analyze' followed by choosing 'built-in analyses'. Then select 'Normalize' from the list. To normalize between 0 and 100%, define zero as the smallest value in each data set, the value in the last row of each data set, or to a value you enter. Define one hundred as the largest value in each data set, the value in the first row in each data set or a value you enter. Check 'present results as: Percentages' and 'create a new graph of results'.
- 11. Go to the newly created graph.
- 12. Click 'Analyze', choose nonlinear regression, choose the panel of equations "Dose-response curves - Inhibition" and then choose the equation "log (inhibitor) vs. response".

\*You might also want to consider constraining the top and bottom of the slope to a constant value.

## 5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

#### 6.0 Spillages -

Always clean up any spills immediately after use - only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.



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## 7.0 Training -

## 8.0 Related documents –

- 8.1 Risk assessments –
- 8.2 SOP SASoM-EQUIP-029-Biohit BP800 Microplate Reader

# 9.0 Approval and sign off –

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