



HC50 and CC50 Calculation

Introduction

This document outlines MAP's method for calculating HC50 (Hemolytic Concentration 50) and CC50 (Cytotoxic Concentration 50) values.

Methodology

Step 1: Average Positive and Negative Absorbance Values

$$\begin{aligned}\text{Average Positive Control} &= \frac{\sum \text{Absorbance at positive control wells}}{\text{Total number of positive control wells}} \\ \text{Average Negative Control} &= \frac{\sum \text{Absorbance at negative control wells}}{\text{Total number of negative control wells}}\end{aligned}$$

Step 2: Absorbance Range

$$\text{Absorbance Range} = \begin{cases} \text{Average Positive Control} - \text{Average Negative Control}, & \text{if assay} = \text{HC50} \\ \text{Average Negative Control} - \text{Average Positive Control}, & \text{if assay} = \text{CC50} \end{cases}$$

Step 3: Absorbance Threshold

$$\text{Absorbance Threshold} = \begin{cases} \text{Average Negative Control} + 0.50 \times \text{Absorbance Range}, & \text{if assay} = \text{HC50} \\ \text{Average Positive Control} + 0.50 \times \text{Absorbance Range}, & \text{if assay} = \text{CC50} \end{cases}$$

Step 4: Determine HC50/CC50 Value

For each sample, moving from the lowest concentration to highest concentration, identify the well that:

- For HC50:
$$\text{Absorbance value} \geq \text{Absorbance Threshold}$$
- For CC50:
$$\text{Absorbance value} \leq \text{Absorbance Threshold}$$

The concentration of the first well that meets the requirements above is identified as the HC50 or CC50 value. If no well meets the criteria, then:

- The HC50/CC50 value is considered to be greater than the highest tested concentration and is denoted as $> i$, where i is the starting concentration in the assay.