

## Trypsin Inhibition Test for Screening Compounds for Potential Drug Development

X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X
X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X
X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X
X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X
X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X
X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X
X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X
X	C	C <sub>h</sub>	S1	S1 <sub>h</sub>	...	...	...	...	Sn	Sn <sub>h</sub>	X

### Notes on plate:

There are only 10 test columns in this plate. The sample columns (S) contain different concentrations of the test compound. You can test up to four concentrations on each plate.

(A standard compound is included in every plate run to verify the integrity of the procedure. The activity level for the standard compound is known.)

### Notes on layout:

In all plate layouts, there are no samples included in the wells at either end of the plate because of potential edge effects (e.g., thermal effects).

In this plate layout “C” represents the control column of the plate.

In the Trypsin test, the wells in the column include the enzyme, but no test compound is added.

In this plate layout “Cb” represents the control blank column of the plate.

In the Trypsin test, the wells in this column only include CaCl solution. No Trypsin is present nor is a test compound added.

In this plate layout “S1” represents the sample column of the plate for the first test concentration. (“Sn” represents the sample column with the last test concentration to be tested.)

In the Trypsin test, the wells in this column include the enzyme and the test compound (extract).

In this plate layout “S1b” represents the sample blank column of the plate for the first test concentration.

In the Trypsin test, the wells in this column only include the test compound and the CaCl solution. No Trypsin is present.

### Calculation procedure for Trypsin activity level:

1. Trypsin activity [%] =  $[(Es-Esb) / (Ec-Ecb)] \times 100$

- Es = Extinction of sample containing Trypsin.
- Esb = Extinction of sample blank containing compound with no Trypsin.
- Ec = Extinction of control containing Trypsin (without compound).
- Ecb = Extinction of control blank containing without Trypsin or compound.
- (Ec-Ecb) = 100% Trypsin activity without inhibition.
- (Es-Esb) = Trypsin activity without inhibitory effect of compound.

2. Activity observations are averaged across replicates for a specific concentration (e.g., S1).
3. The variance in activity level is calculated along with the coefficient of variation.
4. Dixon's test for outliers is applied to remove extreme observations. (The magnitude difference between the two lowest or highest observations is determined. This difference is considered in the presence of the range of the data (the difference between the lowest and highest observations). The ratio is compared with tabled criterion values, dependent upon sample sizes.
5. The mean normalized activity level from the above equation is graphically displayed.
6. A dose-response curve of a standard compound is generated → Determination of IC50 by statistic program "Origin" to verify the integrity of the procedure.
7. Calculation of Z'-Factor (quality criteria)