pyTecanEx program version 1 – User Guide

Table of Contents

Introduction and Motivation

This Python-based program aims to assist all users who obtain results from the Tecan microplate reader. Generally, users who are also scientists usually design experiments using a 96-well plate in a column-based manner, as shown in **Figure 1** below. However, Tecan's microplate reader provides a row-based result. Therefore, additional data organization is required before users can analyze and interpret their data. Some may argue that the users may alter the experimental design corresponding to the microplate reader style, which can solve the problem quickly—this argument is solid. However, this solution is only suitable for an experiment that requires fewer test solutions. For example, like IC50 (half inhibitory concentration) determination, MIC (minimum inhibition concentration), or MBC (minimum bactericidal concentration), these assays require more concentration of the compound of interest. Therefore, the adopted experimental design in a row-based manner is unsuitable since the 96-well plate has more columns, 12, than rows, 8 (as shown in Figure 1 below), and this is the reason why this program was created to assist users in Tecan's reader result data organization and analysis.

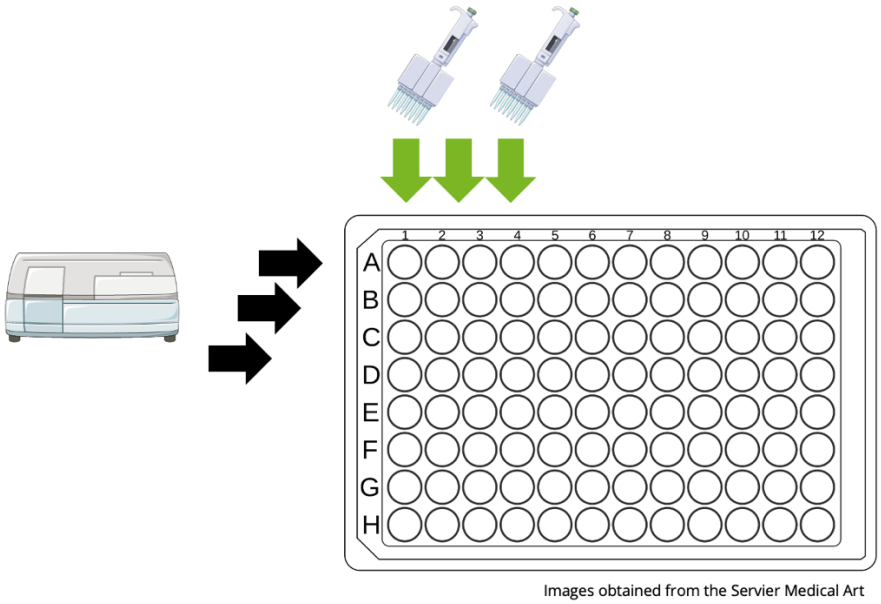

Figure 1. Row- and column-based design used by most users and Tecan's microplate reader.

An essential step before analysis

As mentioned above, the users can use a standard column-based design in their experiment. However, the users should use the **last column** for **control**. In this way, pyTecanEx will organize the users' results obtained from Tecan's microplate reader machine and calculate the percentage activity and percentage inhibition corresponding to the control. The following section provides an example of how to use the program effectively.

Example

Experimental assay:

- IC50 determination of an antibiotic drug against bacteria

Experimental explanation:

- Eleven antibiotic drug concentrations were co-cultivated with bacteria at 37 degrees for 15 hours. The bacterial growth curve was monitored every hour at 600 nm. The obtained bacterial growth from eleven concentrations of antibiotic drugs was compared to control bacterial growth without the drug in the last column. Therefore, there were twelve test samples in total (columns 1 to 12), as shown in **Figure 2**.

Pipette scheme:

- Eleven contractions of an antibiotic drug were added to columns 1 to 11, and the control, without antibiotics, was added to column 12.
- Each antibiotic concentration was repeated three times in rows A to C.
- The pipette scheme is provided in **Figure 2** below.

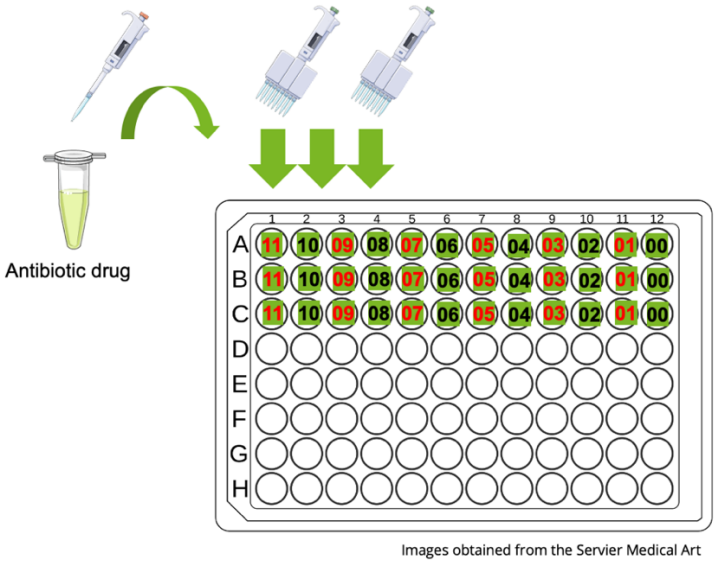

Figure 2. Experimental design and pipette scheme of IC50 determination of antibiotic drug against bacteria.

Prepared obtained data before using the pyTecanEx program:

1. Only the absorbance value should be collected from the Tecan microplate reader data file, as shown in **Figure 3**, highlighted in green.
2. The collected data should be pasted into a new Excel file and saved.
3. In a new Excel file, new cycle times must be added manually above the collected data from step 2, as highlighted in orange.

Tecan's original data **Collected data with added time cycles**

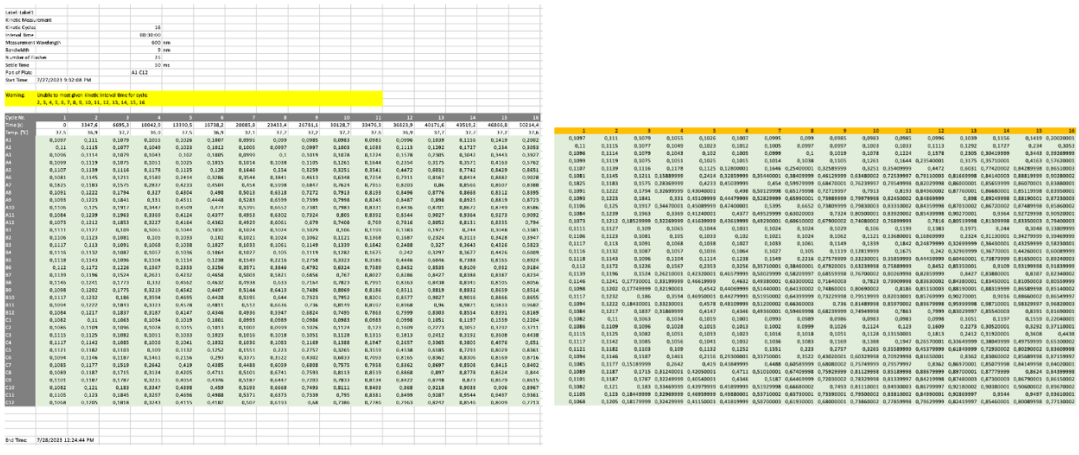

Figure 3. Data collection from Tecan's original data file and saved new data file with manually added cycle times.

Run the program:

- There are seven simple steps to run the program.
    1. Double-click the pyTecanEx icon on your desktop, as shown in **Figure 4**. Alternatively, the users can call the program using a terminal using the code here (python pyTecanEx.py).
    2. Accept the software license agreement, refusing the agreement will cause the program termination.
    3. Enter the cycle number corresponding to the new Excel file saved in the above section. In this example, the cycle number is 16.
    4. Enter the column number corresponding to the new Excel file saved in the above section. In this example, the cycle number is 12.
    5. Click Upload file and select the new Excel file saved in the above section.
    6. Click Execute to run the program.
    7. Click Save to save the program outcomes.

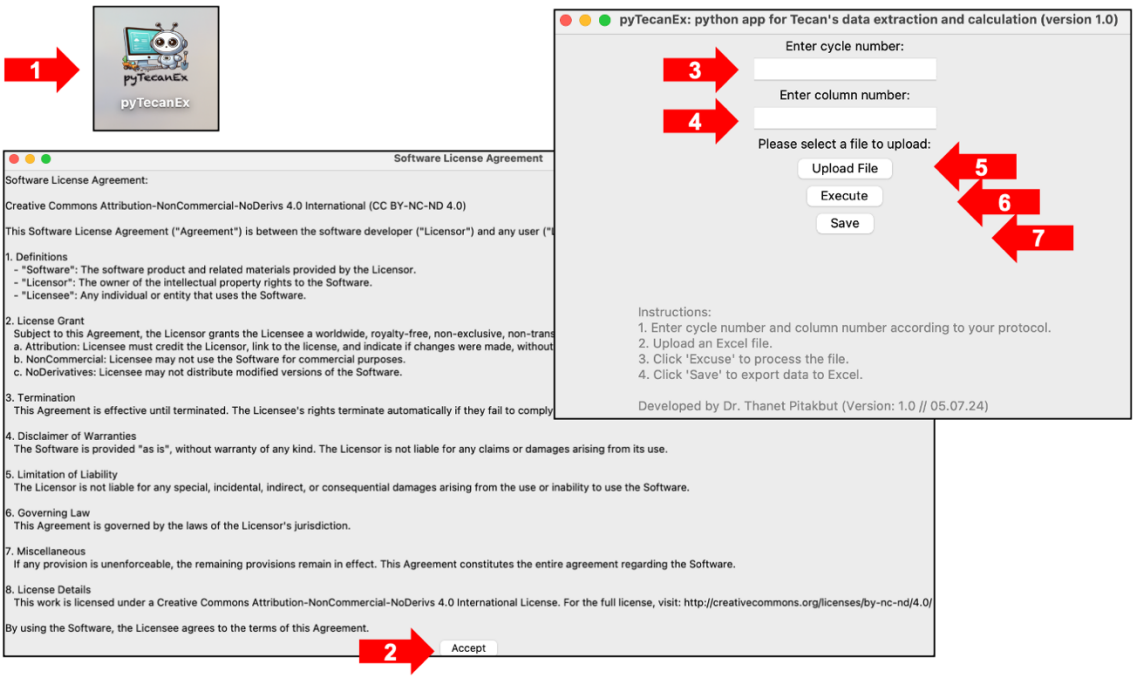

Figure 4. Instructions on how to run the pyTecanEx program, which consists of seven simple steps.

Understand the outcomes:

- Five categories of outcomes will be generated from pyTecanEx, as shown in **Figure 5** and below.

```
basecor_cycle_1.xlsx          log_2024-07-05_12-14-48.txt ori_cycles_std.xlsx          percent_int_10.xlsx
basecor_cycle_10.xlsx         ori_cycle_1.xlsx            percent_act_10.xlsx          percent_int_11.xlsx
basecor_cycle_11.xlsx         ori_cycle_10.xlsx           percent_act_11.xlsx          percent_int_12.xlsx
basecor_cycle_12.xlsx         ori_cycle_11.xlsx           percent_act_12.xlsx          percent_int_13.xlsx
basecor_cycle_13.xlsx         ori_cycle_12.xlsx           percent_act_13.xlsx          percent_int_14.xlsx
basecor_cycle_14.xlsx         ori_cycle_13.xlsx           percent_act_14.xlsx          percent_int_15.xlsx
basecor_cycle_15.xlsx         ori_cycle_14.xlsx           percent_act_15.xlsx          percent_int_16.xlsx
basecor_cycle_16.xlsx         ori_cycle_15.xlsx           percent_act_16.xlsx          percent_int_2.xlsx
basecor_cycle_2.xlsx          ori_cycle_16.xlsx           percent_act_2.xlsx           percent_int_3.xlsx
basecor_cycle_3.xlsx          ori_cycle_2.xlsx            percent_act_3.xlsx           percent_int_4.xlsx
basecor_cycle_4.xlsx          ori_cycle_3.xlsx            percent_act_4.xlsx           percent_int_5.xlsx
basecor_cycle_5.xlsx          ori_cycle_4.xlsx            percent_act_5.xlsx           percent_int_6.xlsx
basecor_cycle_6.xlsx          ori_cycle_5.xlsx            percent_act_6.xlsx           percent_int_7.xlsx
basecor_cycle_7.xlsx          ori_cycle_6.xlsx            percent_act_7.xlsx           percent_int_8.xlsx
basecor_cycle_8.xlsx          ori_cycle_7.xlsx            percent_act_8.xlsx           percent_int_9.xlsx
basecor_cycle_9.xlsx          ori_cycle_8.xlsx            percent_act_9.xlsx           percent_int_means.xlsx
basecor_mean_cycles.xlsx      ori_cycle_9.xlsx            percent_act_means.xlsx       percent_int_stds.xlsx
basecor_std_cycles.xlsx       ori_cycles_mean.xlsx        percent_act_stds.xlsx
```

Figure 5. Outcomes obtained from a successful run from pyTecanEx

- The first category is **ori_cycle_xx**

  (Original data in each cycle)

  In this category, pyTecanEx helps users extract, organize, and adapt the original data obtained from Tecan's microplate reader in a row-to-column-based manner. Furthermore, pyTecanEx automatically calculates each test sample's average and standard deviation. Finally, **ori_cycle_mean** and **ori_cycle_std** extract each sample's mean and standard deviation each time in a single file. This will give a ready-to-use file for users to plot a scatter plot using Excel, as shown in **Figure 6**.

	Antibiotic Concentration											Control
Time	11	10	9	8	7	6	5	4	3	2	1	0
0	0,109667	0,109733	0,110933	0,111067	0,111533	0,109833	0,134967	0,110867	0,109733	0,110167	0,109433	0,1075
1	0,111233	0,111567	0,1123	0,1131	0,115467	0,115433	0,118533	0,121667	0,1204	0,123067	0,123033	0,121133
2	0,107733	0,108467	0,1084	0,108233	0,1105	0,1208	0,153933	0,176067	0,1801	0,1869	0,188367	0,1836
3	0,105133	0,104233	0,1054	0,105467	0,1124	0,1539	0,27	0,3238	0,325133	0,346267	0,332967	0,3219
4	0,102967	0,102367	0,103033	0,1034	0,112367	0,230767	0,421833	0,439033	0,4369	0,4534	0,4466	0,4142
5	0,1013	0,1015	0,101833	0,1037	0,125667	0,315733	0,451567	0,477433	0,440033	0,4586	0,472533	0,429667
6	0,1004	0,100933	0,1016	0,102567	0,1582	0,349667	0,4677	0,501733	0,520467	0,5261	0,5148	0,497833
7	0,1001	0,100667	0,102633	0,1057	0,232867	0,373633	0,595933	0,652967	0,648633	0,658667	0,6437	0,6067
8	0,1	0,102833	0,1073	0,1131	0,292467	0,4569	0,6837	0,7343	0,736267	0,7399	0,7341	0,680467
9	0,100867	0,108267	0,118167	0,131033	0,327967	0,6235	0,7623	0,794967	0,796667	0,801533	0,804967	0,742967
10	0,105567	0,121033	0,146033	0,175533	0,3562	0,7312	0,798	0,823433	0,818833	0,834167	0,845667	0,777967
11	0,112567	0,146967	0,195967	0,2477	0,448533	0,8176	0,828367	0,857567	0,847333	0,856433	0,867033	0,7926
12	0,135367	0,1963	0,266233	0,3279	0,608733	0,835467	0,857467	0,8728	0,8849	0,8982	0,930467	0,8199
13	0,159767	0,263067	0,329233	0,368433	0,747433	0,860967	0,848733	0,8597	0,8829	0,890867	0,9593	0,841033
14	0,200867	0,302	0,379233	0,452167	0,820767	0,892367	0,846967	0,841367	0,8719	0,889167	0,953433	0,8245
15	0,2529	0,357033	0,472933	0,609367	0,864533	0,8976	0,834133	0,8297	0,861733	0,8736	0,937833	0,794067

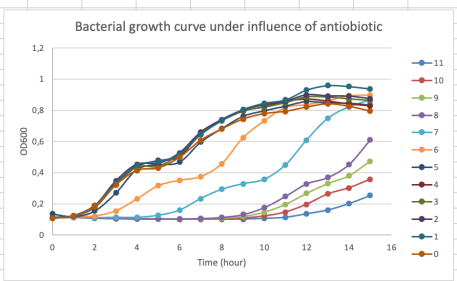

Figure 6. An Excel sheet and a scatter plot obtained from an **ori_cycle_mean** file.

- The second category is **basecor_cycle_xx**

  (Baseline correction of each cycle)

  This category provides the users with a result of each sample's mean and standard deviation deducted each time with the first-time measurement. Like the first category, **basecor_cycle_mean** and **basecor_cycle_std** extract each sample's mean and standard deviation each time in a single file. This will again give a ready-to-use file for users to plot a scatter plot using Excel, as shown in Figure 7.

	Antibiotic Concentration											Control
Time	11	10	9	8	7	6	5	4	3	2	1	0
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0,001567	0,001833	0,001367	0,002033	0,003933	0,0056	-0,01643	0,0108	0,010667	0,0129	0,0136	0,013633
2	-0,00193	-0,00127	-0,00253	-0,00283	-0,00103	0,010967	0,018967	0,0652	0,070367	0,076733	0,078933	0,0761
3	-0,00453	-0,0055	-0,00553	-0,0056	0,000867	0,044067	0,135033	0,212933	0,2154	0,2361	0,223533	0,2144
4	-0,0067	-0,00737	-0,0079	-0,00767	0,000833	0,120933	0,286867	0,328167	0,327167	0,343233	0,337167	0,3067
5	-0,00837	-0,00823	-0,0091	-0,00737	0,014133	0,2059	0,3166	0,366567	0,3303	0,348433	0,3631	0,322167
6	-0,00927	-0,0088	-0,00933	-0,0085	0,046667	0,239833	0,332733	0,390867	0,410733	0,415933	0,405367	0,390333
7	-0,00957	-0,00907	-0,0083	-0,00537	0,121333	0,2638	0,460967	0,5421	0,5389	0,5485	0,534267	0,4992
8	-0,00967	-0,0069	-0,00363	0,002033	0,180933	0,347067	0,548733	0,623433	0,626533	0,629733	0,624667	0,572967
9	-0,0088	-0,00147	0,007233	0,019967	0,216433	0,513667	0,627333	0,6841	0,686933	0,691367	0,695533	0,635467
10	-0,0041	0,0113	0,0351	0,064467	0,244667	0,621367	0,663033	0,712567	0,7091	0,724	0,736233	0,670467
11	0,0029	0,037233	0,085033	0,136633	0,337	0,707767	0,6934	0,7467	0,7376	0,746267	0,7576	0,6851
12	0,0257	0,086567	0,1553	0,216833	0,4972	0,725633	0,7225	0,761933	0,775167	0,788033	0,821033	0,7124
13	0,0501	0,153333	0,2183	0,257367	0,6359	0,751133	0,713767	0,748833	0,773167	0,7807	0,849867	0,733533
14	0,0912	0,192267	0,2683	0,3411	0,709233	0,782533	0,712	0,7305	0,762167	0,779	0,844	0,717
15	0,143233	0,2473	0,362	0,4983	0,753	0,787767	0,699167	0,718833	0,752	0,763433	0,8284	0,686567

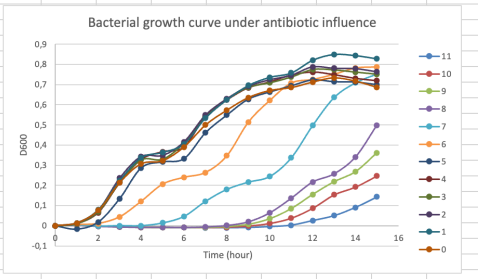

Figure 7. An Excel sheet and a scatter plot obtained from a **basecor_cycle_mean** file.

- The third and fourth categories are **percent_act_xx** and **percent_int_xx**

  (Percent activity and percent inhibition of each cycle)

  These categories are normalized results of each test sample's value each time corresponding to the control, with the maximum growth activity of 100% and maximum growth inhibition of 100%. To calculate the IC50 value of the antibiotic of interest, the user can select any preferable time point to calculate. Figure 8 below shows a classic example of using an antibiotic concentration-response curve to determine the IC50 value of the antibiotic of interest with a simple linear regression.

Cycle 16	11	10	9	8	7	6	5
	0,86821	0,715596	0,587738	0,320955	-0,09859	-0,15727	0,04427
	0,679605	0,599012	0,33578	0,309386	-0,10176	-0,13818	-0,00141
	0,831151	0,604966	0,499925	0,188412	-0,08954	-0,14703	-0,10113
Mean	0,792989	0,639858	0,474481	0,272918	-0,09663	-0,14749	-0,01942
Standard D	0,08159	0,05361	0,104423	0,059941	0,005178	0,007802	0,06071

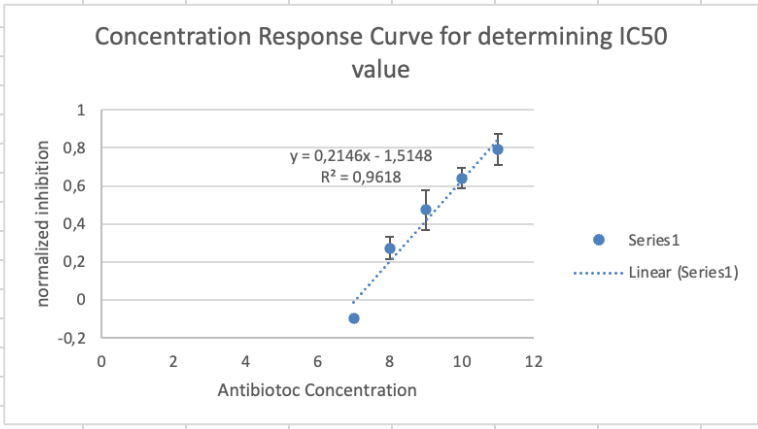

Figure 8. An Excel sheet and a scatter plot with a simple linear regression obtained from the percent_int_16 file.

- The last category is **a log file**.

  This file will inform the users whether the program can run successfully or not. If there is any error, the user will find it on this log file.

Contact information

For further inquiries, please get in touch with Dr. rer. nat. Thanet Pitakbut via email (thanet.pitakbut@fau.de).