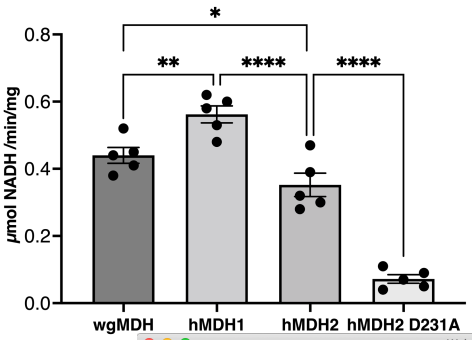
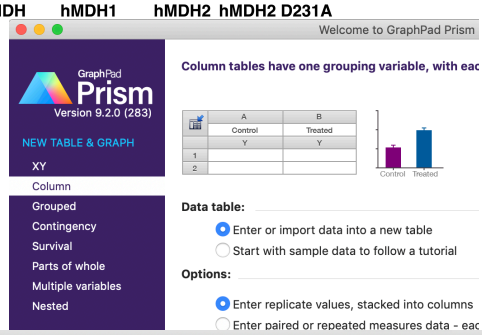
Biochemistry Lab

Graph Pad MDH Instructions

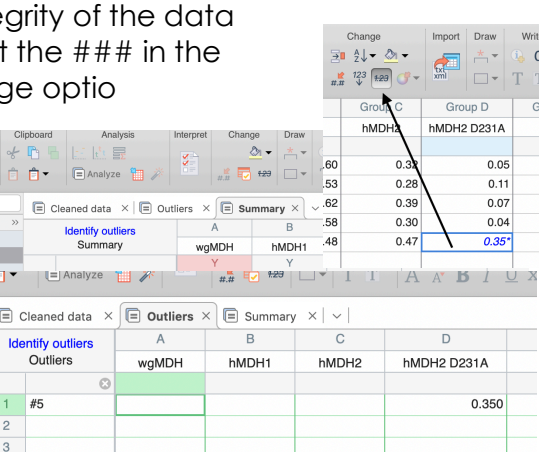
Specific Activity Graph: The final product should look like the specific activity example graph shown here. The data is not real nor even close to the values you may get – they are purposefully made up from random values. However it example is to show the expected format and style for your graphs when using graph pad/prism software.

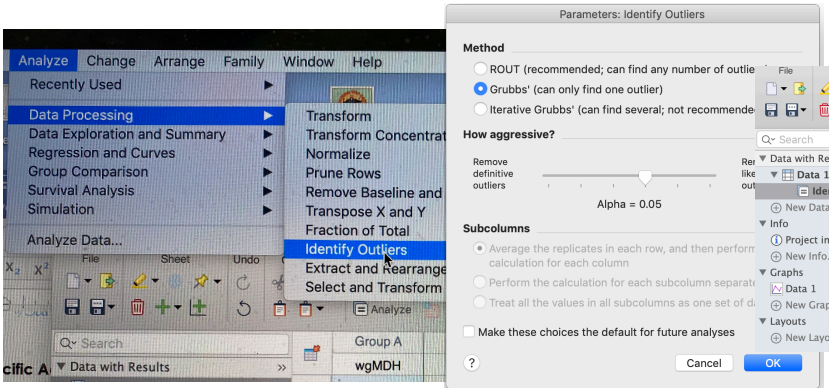


- Start by creating an new column table and graph. There are two options. One where you add all data or another option when you've processed the data to create an average and calculated the statistical measurement (STD DEV or SEM) ahead of time.
  - Your data are 4 to 6 different measurements (1 ml assay) without relationship to other measurements. Thus your data are NOT paired. So you will Enter replicate values (repeats from each enzyme assay) stacked into columns. Click on the options as shown and click "create"



- Enter the name of the enzyme in the title portion of the graph (not in cells for data), then in column format, enter the specific activity of each assay you performed.
- Now check to see if there are outlier data points present.
  - First in the main pull-down menu, select analyze -> Data Processing -> Identify Outliers.
  - Then an Analyze Data window will pop up, select the columns you wish to have analyzed (one to all columns of data) and then select "OK"
  - There are two main methods available in GraphPad/Prism. Grubbs and ROUT. Grubbs is to be used to remove only ONE data point per column. ROUT is a method to test for multiple outliers. For this experiment with the smaller numbers, select Grubbs and use the most commonly used alpha value of 0.05.
  - A new window with tabs under the file "Identity Outliers" will appear and you can, by clicking on the tabs see if there is an outlier and which data point is the outlier.
  - There are two options at this point. Use the "cleaned up data" OR and the PREFERED method, is to "mask or hide" the data point without deleting. This operation will hide the outlier from the graph and calculations but keep the integrity of the data without deletion.





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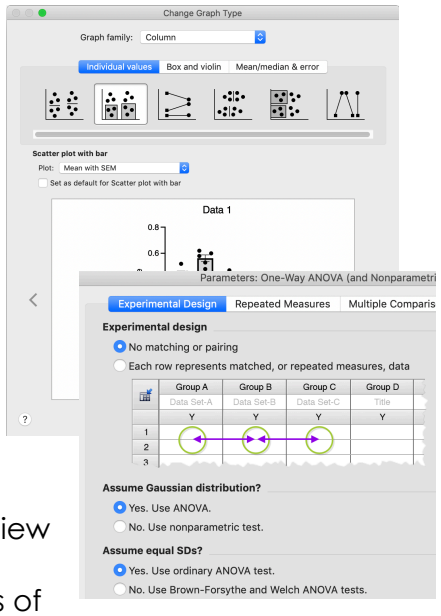
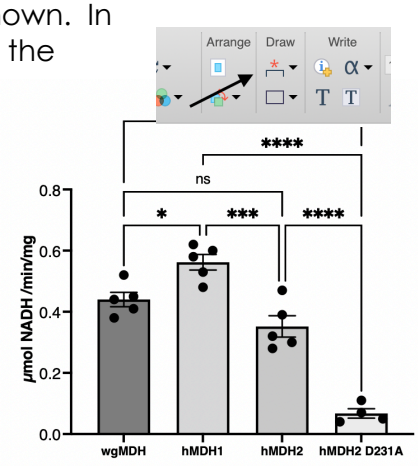
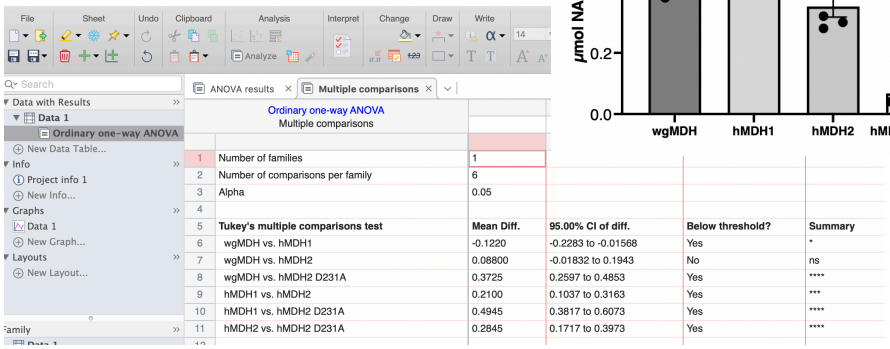
- Click on the graph icon on the left side of the table and a pop up window will give you options for your graph the first time you enter data.
  - Select Column and a "bar graph" and select Mean with SEM (standard error of the mean) then click "OK"
  - In the new window, adjust the axis, remove the " X title" and "Data" text by highlighting and deleting the text.
  - Correct the text for the units of specific activity (see example graph above).



- Statistical Analysis. To compare each mean with each of the other means a one-way ANOVA is used to compare the means of three or more unmatched groups (which is what this type of experiment is). We assume the population follow a Gaussian distribution and that all of the samples have the same standard deviation (same variance) so a simple ANOVA can be used. Review the information in the statistics handout for background on null hypothesis and why a T test is not appropriate to compare means of more than one set of data.
  - Ensure that "no matching or pairing", "Yes use ANOVA", and "…Ordinary ANOVA…" are selected.
  - Then click on the "Multiple Comparisons" box at the top of the popup menu.
  - Because there is no control in this test, we want to compare all means to all other column means, so select "Compare the mean of each column with the mean of every other column" in the Multiple Comparisons popup window. Click "OK".
  - A new result table will show up on the left side of the Prism window "Ordinary one-way ANOVA. Look for the "Multiple Comparisons" tab and click.
  - There is a lot of information there, but for now, focus on the Tukey's test using an alpha value of 0.05. In the first column each column mean being compared is shown. In the summary you will see 1-4 "****" or ns (for not significant). Refer to the statistics handout to understand the meaning and value of the p value stars/ns. GraphPad also has a great statistics help section if you want more information.
    - Click on the graph icon to show the graph then click on the icon for brackets with statistical information. Brackets with "stars" will appear.
    - Click to remove comparison brackets that are not needed.
    - Export as a PNG or JPEG file for your lab book/presentation.





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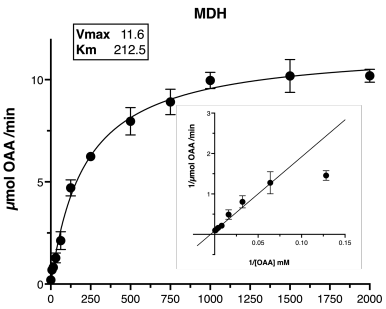
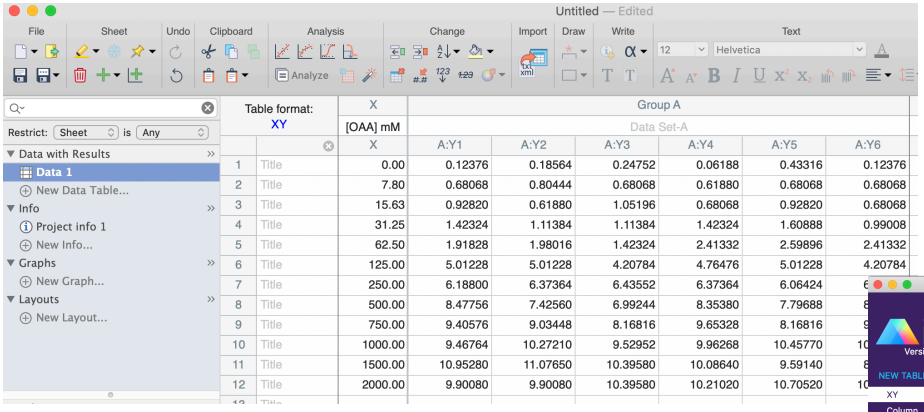
Graph Pad MDH Instructions

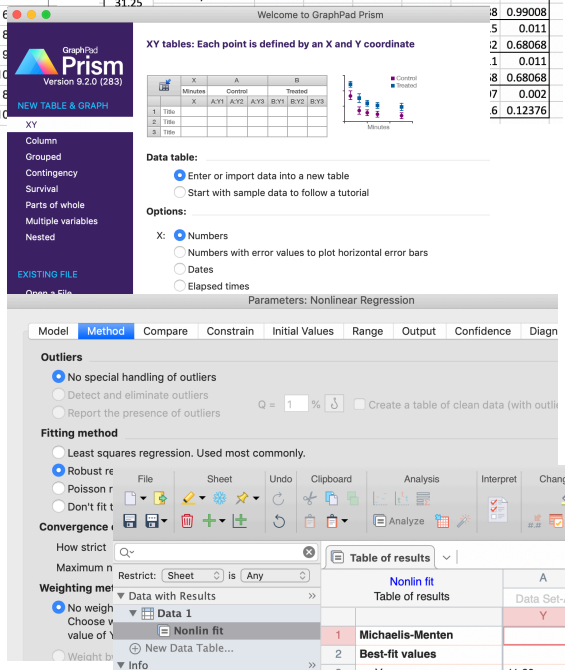
Michalis Menten and Lineweaver Burke (Km & Vmax) Graphs: As above, the data provided for this example is made up and random numbers used. Calculate the enzyme activity (µmol substrate/min) based on the information in the handout on enzyme assays for the 96 well assay.



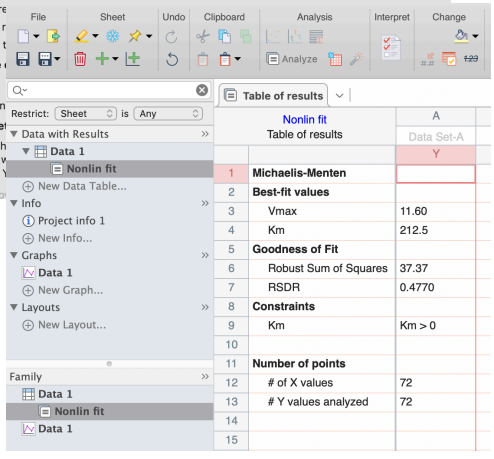
- Set up the change in absorbance and perform the conversion to enzyme activity (rate) in a table of rows as shown here.
- Open a new file (XY) table in prism.
  - Select enter 6 replicate Y values and leave the X as Numbers.
- Enter the data as seen. Do this for each wild-type MDH and mutant. If you are working on an inhibition each group will be entered for the various inhibitor concentrations.



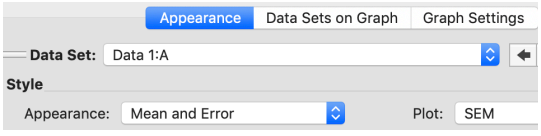
[OAA]		[OAA] mM					
2000	Abs/min	0.16	0.16	0.168	0.165	0.173	0.162
	µmol NADH/min	9.9008	9.9008	10.3958	10.2102	10.7052	10.0246
1500	Abs/min	0.177	0.179	0.168	0.163	0.155	0.145
	µmol NADH/min	10.9528	11.0765	10.3958	10.0864	9.5914	8.9726
1000	Abs/min	0.153	0.166	0.154	0.161	0.169	0.163
	µmol NADH/min	9.46764	10.2721	9.52952	9.96268	10.4577	10.0864
750	Abs/min	0.152	0.146	0.132	0.156	0.132	0.146
	µmol NADH/min	9.40576	9.03448	8.16816	9.65328	8.16816	9.03448
500	Abs/min	0.137	0.12	0.113	0.135	0.126	0.141
	µmol NADH/min	8.47756	7.4256	6.99244	8.3538	7.79688	8.72508
250	Abs/min	0.1	0.103	0.104	0.103	0.098	0.097
	µmol NADH/min	6.188	6.37364	6.43552	6.37364	6.06424	6.00236
125	Abs/min	0.081	0.081	0.068	0.077	0.081	0.068
	µmol NADH/min	5.01228	5.01228	4.20784	4.76476	5.01228	4.20784
62.5	Abs/min	0.031	0.032	0.023	0.039	0.042	0.039
	µmol NADH/min	1.91828	1.98016	1.42324	2.41332	2.59896	2.41332
31.25	Abs/min	0.023	0.018	0.018	0.023	0.026	0.016



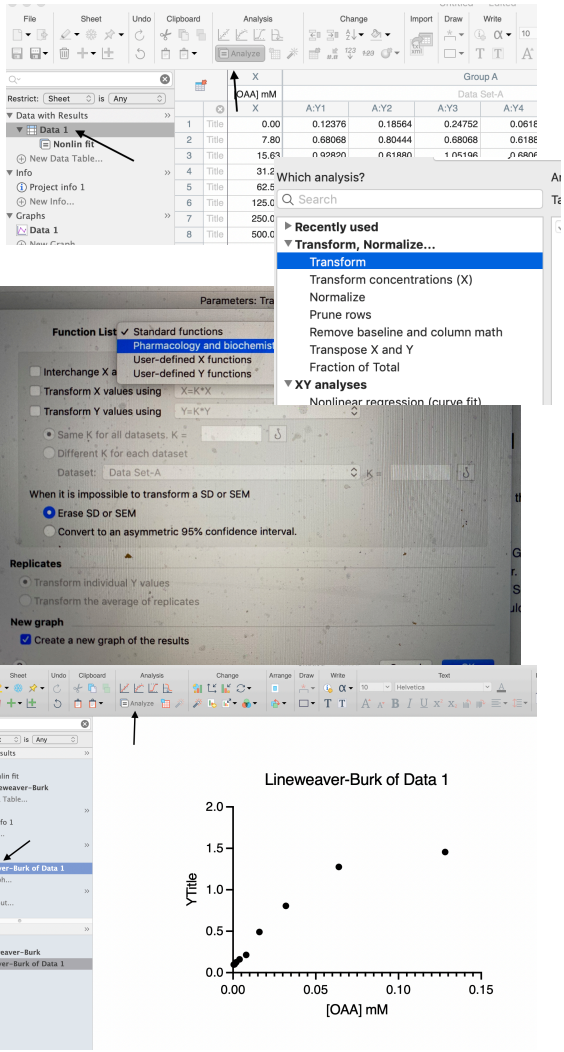
- Click on Analyze to start creating the MM graph. Under XY analysis, select "Nonlinear regression (curve fit)", click OK.
- Scroll down in the "model" tab of the Parameters popup window and click on "Enzyme kinetics-Velocity as a function of substrate" to expand. You should see the M-M option. DO NOT CLICK OK
- In the menu bar of the popup window, click on and open the "Method". You can use the online Grubbs calculator to determine if you have outliers. Another option is to select the robust regression. This option will peform a basic Grubbs analysis and ignores them for your curve.
- NOW click OK. You should see a new file on the right side called Nonlin fit. This is where the table of results including your Km and Vmax values will be displayed.



- On the left will be a graph icon with the label "Data 1" This is the basic MM graph. If you didn't select earlier, you can click on one of the data points to change the appearance from Mean to Mean and Error – use the SEM option.

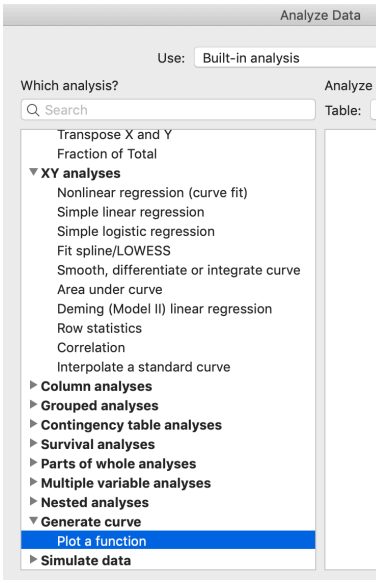


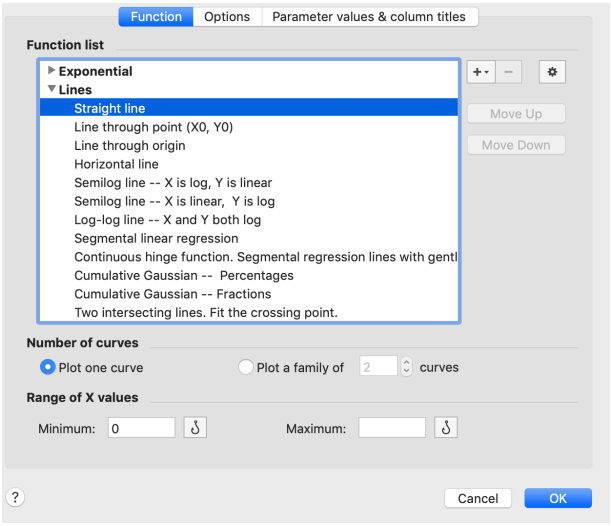
- Adjust your graph to look similar to the example graph above (except the insert graph).



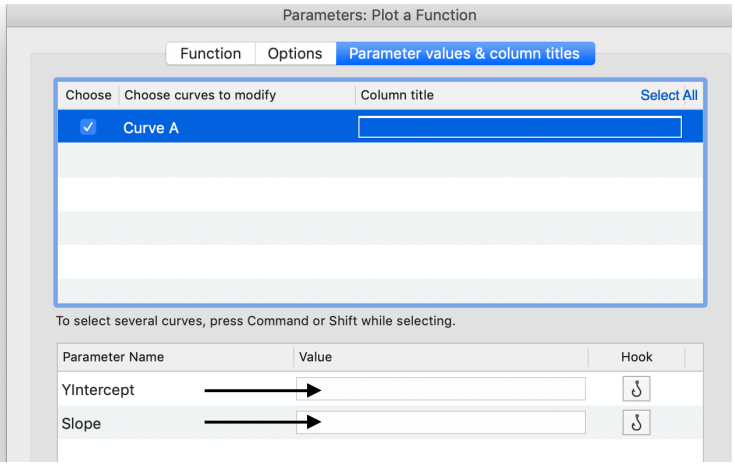
<u>Create the Lineweaver-Burk plot for the insert.</u>

- Record the Km and Vmax from the Nonlit fit table on a scratch pad.
- Click on the data table "Data 1" in the upper left side of the program. Click the "Analyze" button in the toolbar.
- Under "Transform, Normalize…" option, click on "Transform" and <u>*click OK*</u>.
- In the "Function List" dropdown menu, select "Pharmacology and biochemistry transforms".
- Select the "Lineweaver-Burk" option, you will see several secondary plot options including Lineweaver-Burk. For replicates, select "Transform individual Y values, and "Create new graph of the results" then click OK.
- Add the appropriate line to the Lineweaver-Burk graph.
  - From the graph of the transformed data, click the Analyze button in the Analysis section of the toolbar
  - Scroll down to the "Generate curve" section of analyses, select "Plot a function", and click OK
  - Expand "Lines" and click on "Straight line" from the "Function" tab.
  - Use the "Range of X values" options at the bottom of the Function tab to specify where the line should start and end. Select the higher number of the X axis of the Lineweaver-Burk graph. For this example only use 0.15.





- Switch to the "Parameter values & column titles" tab
- Find the values recorded on scratch paper for Vmax and Km, then calculate 1/Vmax and enter this value as the Y Intercept (where Vmax is the value reported by nonlinear regression earlier)
- Enter your calculated Km/Vmax as the Slope.
- Click OK



- Adjust the axis lable and settings to match that shown above.
- Under "File" export the image as a PNG and then insert into your M-M graph for the final figure.