BIOCHROM EZ READ 400 MICROPLATE READER

TECH TIPS: USING A STANDARD CURVE TO PREDICT THE CONCENTRATIONS OF UNKNOWNS

Data can be easily exported from ADAP Basic into Excel for analysis. Many laboratory experiments are quantitative tests which use a standard curve to predict the concentration of unknown samples. This technical tip guides the user from gathering and exporting data using ADAP into Excel in order to determine unknown concentrations.

1. Connect instrument to a power source using the appropriate power cord and power supply. Switch on instrument.
   - ✓ Check the user's manual for important safety information.

2. Connect the instrument to a PC**:** Ensure that you first have administrator rights for installing the software and connecting the instrument to the PC for the first time. Connect to the PC to the instrument using the USB port on the PC to the USB port on the back of the instrument using the supplied USB A to B cable. Determine the communication port (COM) used by the instrument. In the **Start** menu of the PC, go to **Control Panel\System\Hardware\Device Manager\Ports** to determine the COM port used by the instrument. If you are unsure which COM port is being used, please disconnect and connect the USB cable and observe which COM port reappears after re-connecting to the PC.

   Please Note: The instrument cannot connect to the PC if a COM port higher that 9 is used. If this is the case please use the supplied COM port reassignment utilty to reassign the COM port to any unused port from 1-9.

3. Insert the CD supplied with the instrument into the PC that will be used to control the instrument. Install ADAP. Once the program is installed, open ADAP. ADAP will prompt for a user ID and password. For the first time the program is used, enter the pre-set ID and passwords: **sadmin\sadmin**.

4. Once logged as **sadmin**, the **change password** button will appear. Select this option to set specific user IDs, passwords and administrative rights.

Figure 1 ADAP Login

Configure the login and users by entering in the user name and password and the level of administrator rights.

Level 1 (user) can use ADAP for perform quick measurements or use test definitions to acquire and analyze data.

Level 2 (administrator) can perform all basic measurements, create new test definitions for data collection and analysis and can configure system and instrument parameters.

Level 3 (system administrator) has the same privileges as levels 1 and 2 as well as the ability to add, delete or edit users.

5. Select **Setup>Instrument** in the menu bar. A dialogue box will open:

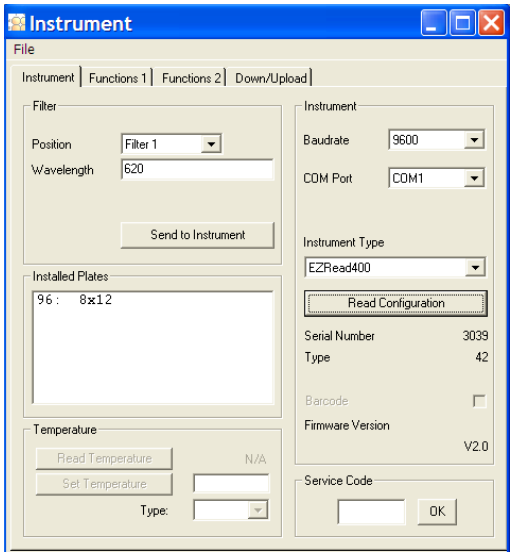


Figure 2 Connect to the Instrument
In the **Instrument** tab, select

- **Baudrate:** select Auto Sense
- **COM** Port: select port as determined in step 2
- **Instrument Type**: select EZ Read 400

Select File>Save to return to main menu and confirm the connection to the instrument.

6. **To measure a plate:**
Go to **Reading/Quick** or the **R** button in the menu bar.

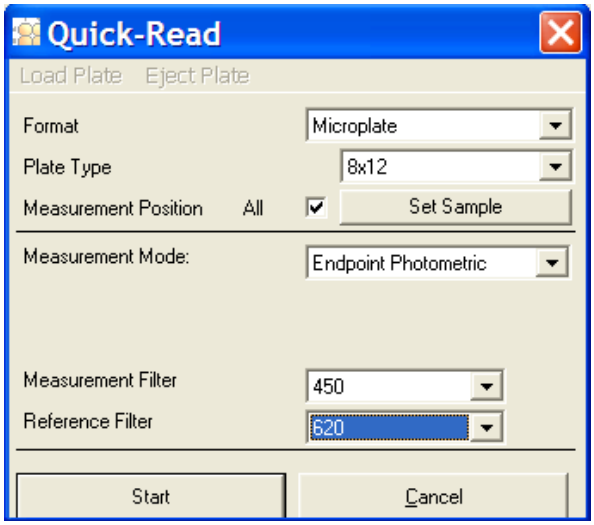


Figure 3 Quick Read Dialogue Box
In the **Quick-Read** dialogue box:

- Confirm that the correct format and plate type are selected.
- Select **All** in **Measurement Position** to read the entire plate.
- Select **Endpoint Photometric** for basic readings using a measurement and reference filter.
- Select the measurement filter and the reference filter from the drop-down menu.

Note: It is important to use a reference filter to account for optical inference from the plate.

7. Place plate in the plate transporter. Select **Start**. Absorbance measurements will appear in the open matrix in ADAP. When prompted, enter a plate ID.

8. Export the data. Ensure that absorbance measurements are visible in the open matrix. If not, select the OD tab so that the absorbance data can be seen in the open matrix. In the menu bar,

select **Options>Copy all data on to clipboard**. Now open Excel. Select Paste or control (v) to paste into the empty workbook. Data will paste as a matrix with filter wavelength, with time and date.

9. In a new Excel spreadsheet, layout the page with the plate layout and raw data:

Data Export

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.453	1.446	1.398	1.432	0.240	0.085	0.503	0.082	0.086	0.440	0.085	0.091
B	0.729	0.720	0.714	0.615	0.595	0.118	0.082	0.648	0.130	0.088	0.435	0.082
C	0.431	0.432	0.420	0.386	0.087	0.084	0.570	0.129	0.084	0.082	0.084	0.085
D	0.239	0.234	0.235	0.217	0.237	0.334	0.081	0.084	0.090	0.173	0.086	0.088
E	0.146	0.145	0.148	0.156	0.090	0.086	0.081	0.256	0.084	0.085	0.578	0.601
F	0.109	0.115	0.115	0.108	0.508	0.086	0.500	0.083	0.336	0.085	0.092	0.085
G	0.096	0.150	0.099	0.097	0.082	0.337	0.082	0.156	0.414	0.088	0.084	0.088
H	0.088	0.090	0.092	0.086	0.166	0.085	0.634	0.088	0.101	0.091	0.089	0.082

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	S1	1	9	17	25	33	41	49	57
B	S2	S3	S4	S5	2	10	18	26	34	42	50	58
C	S3	S4	S5	S6	3	11	19	27	35	43	51	59
D	S4	S4	S4	S4	4	12	20	28	36	44	52	60
E	S5	S5	S5	S5	5	13	21	29	37	45	53	61
F	S6	S6	S6	S6	6	14	22	30	38	46	54	62
G	S7	S7	S7	S7	7	15	23	31	39	47	55	63
H	Blank	Blank	Blank	Blank	8	16	24	32	40	48	56	64

Standards
Blank
Samples

10. Determine the average absorbance of the blank wells and subtract this value from the remaining wells:

Average Blank Absorbance:

B1	B2	B3	B4	Mean (OD)
0.088	0.09	0.092	0.086	0.089

Blank Corrected Absorbance:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.364	1.357	1.309	1.343	0.151	-0.004	0.414	-0.007	-0.003	0.351	-0.004	0.002
B	0.640	0.631	0.625	0.526	0.506	0.029	-0.007	0.559	0.041	-0.001	0.346	-0.007
C	0.342	0.343	0.331	0.297	-0.002	-0.005	0.481	0.040	-0.005	-0.007	-0.005	-0.004
D	0.150	0.145	0.146	0.128	0.148	0.245	-0.008	-0.005	0.001	0.084	-0.003	-0.001
E	0.057	0.056	0.059	0.067	0.001	-0.003	-0.008	0.167	-0.005	-0.004	0.489	0.512
F	0.020	0.026	0.026	0.019	0.419	-0.003	0.411	-0.006	0.247	-0.004	0.003	-0.004
G	0.007	0.061	0.010	0.008	-0.007	0.248	-0.007	0.067	0.325	-0.001	-0.005	-0.001
H	-0.001	0.001	0.003	-0.003	0.077	-0.004	0.545	-0.001	0.012	0.002	0.000	-0.007

11. Next, compile a table of the blank corrected absorbance values of the standards:

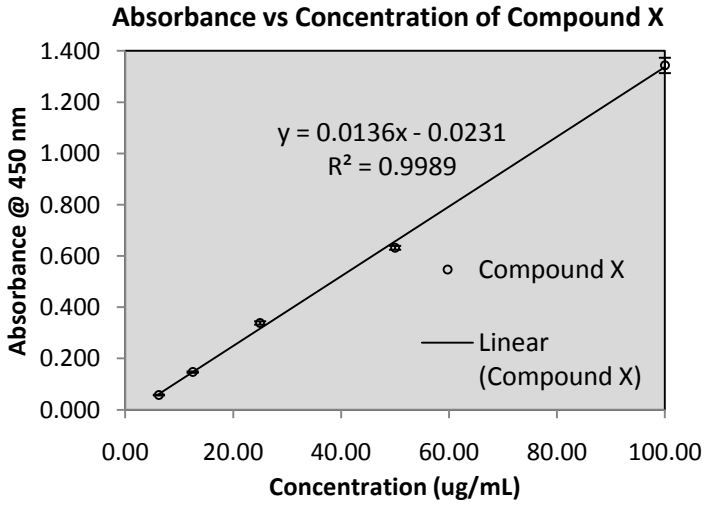
Well Label	Compound X (µg/mL)	1	2	3	4	Mean (OD)	Standard Deviation (OD)	Coefficient of Variation (%)
S1	100.00	1.364	1.357	1.309	1.343	1.343	0.030	2.23%
S2	50	0.640	0.631	0.625	0.526	0.632	0.008	1.19%
S3	25.00	0.342	0.343	0.331	0.297	0.339	0.007	1.97%
S4	12.50	0.150	0.145	0.146	0.128	0.147	0.003	1.80%
S5	6.25	0.057	0.056	0.059	0.067	0.057	0.002	2.66%
S6	3.13	0.020	0.026	0.026	0.019	0.024	0.003	14.43%
S7	1.56	0.007	0.061	0.010	0.008	0.026	0.030	116.72%

For each standard, calculate the mean or average value, standard deviation and coefficient of variation using the following formulas in Excel:

Mean Standard 1 (S1): =AVERAGE(wells A1, A2, A3 and A4)
Standard Deviation S1: =STDEV(wells A1, A2, A3 and A4)
Coefficient of Variation (%CV) =standard deviation/mean*100%

The %CV is a useful metric for determining the reliability of the data. Typically, %CV of <5% suggests that the data is reliable (this assumption is assay type dependent). Thus the mean absorbance values of S6 and S7 will not be included in the standard curve because the %CV is >5% (14.4% and 116.7% respectively).

12. Plot the mean absorbance values of standards S1 – S5 as a function of the known concentrations as a x-y scatter plot:



Fit a linear regression trend line to the data by selecting trend line in the chart layout menu. The example data conforms to the linear trend as represented by the R^2 value: 0.9989 or 99.89%.Thus the equation of the linear regression trend line can be used to determine the concentration of samples

from their absorbance values. The standard deviation from the mean absorbance values were used to plot error bars to the data points

Please note: The standard deviation can be used to fit y-error bars to the data (as shown above).

Please note: Other curve fitting algorithms may be more appropriate to your data such as 4-parameter fit, cubic spline or polynomial regression.

13. The equation of the line is then used to calculate the concentration of the samples by solving for x and inputting the blank-corrected absorbance for the y value:

Concentrations (ug/mL)

	1	2	3	4	5	6	7	8	9	10	11	12
A	101.99	101.48	97.95	100.45	12.80	~~1.40~~	32.14	~~1.18~~	~~1.48~~	27.51	~~1.40~~	~~1.85~~
B	48.76	48.10	47.65	40.38	38.90	3.83	~~1.18~~	42.80	4.71	~~1.63~~	27.14	~~1.18~~
C	26.85	26.92	26.04	23.54	~~1.55~~	~~1.33~~	37.07	4.64	~~1.33~~	~~1.18~~	~~1.33~~	~~1.40~~
D	12.73	12.36	12.43	11.11	12.58	19.71	~~1.11~~	~~1.33~~	~~1.77~~	7.88	~~1.48~~	~~1.63~~
E	5.89	5.82	6.04	6.63	~~1.77~~	~~1.48~~	~~1.11~~	13.98	~~1.33~~	~~1.40~~	37.65	39.35
F	~~3.17~~	~~3.61~~	~~3.61~~	~~3.10~~	32.51	~~1.48~~	31.92	~~1.26~~	19.86	~~1.40~~	~~1.92~~	~~1.40~~
G	~~2.21~~	~~6.18~~	~~2.43~~	~~2.29~~	~~1.18~~	19.93	~~1.18~~	6.63	25.60	~~1.63~~	~~1.33~~	~~1.63~~
H	~~1.63~~	~~1.77~~	~~1.92~~	~~1.48~~	7.36	1.40	41.77	~~1.63~~	~~2.58~~	~~1.85~~	~~1.70~~	~~1.18~~

These concentration values are based on the standard curve, thus only concentrations that are >100 ug/mL or <3.13 ug/mL are considered valid; all other values are disregarded. A small amount of extrapolation may be acceptable depending on the linear range of the instrument and the linear range of the assay.