BIOCHROM ADAP PLUS AND EXPERT SOFTWARE
WRITING A TEST DEFINITION

ADAP is the premier software offering from Biochrom for Anthos Microplate Readers. ADAP Plus and Expert are used for reader control and integrated data analysis.

> **Note**: ADAP Expert has an additional analysis options that are not available in ADAP Plus.

Getting Started: Installing ADAP and connecting to the instrument

1. **To turn on the instrument:**
   Connect instrument to a power source using the power supply. Turn on the instrument using the switch at the back of the instrument.

   Please note: Check user's manual for important safety information.
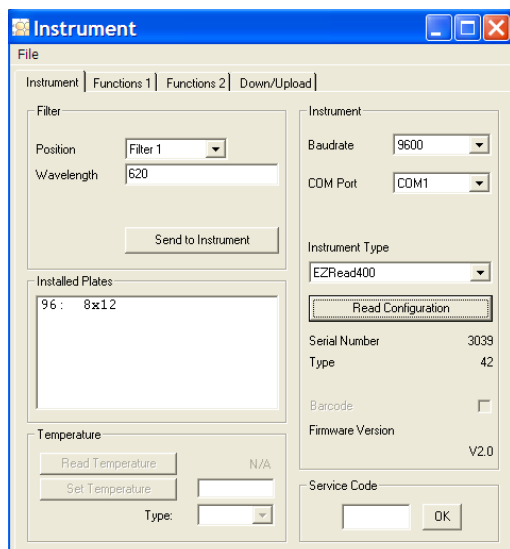
2. **To connect the instrument to a PC:**
   Ensure that you first have administrator rights for installing the software and connecting the instrument to the PC for the first time. Connect to the PC to the instrument using the USB port on the PC to the USB port on the back of the instrument using the 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 utility to reassign the COM port to any unused port from 1-9.

3. **To connect instrument to ADAP 2.0 software:**
   Insert the CD supplied with the instrument into a PC, install ADAP. Open ADAP. ADAP will prompt for a user ID and password. Login using the pre-set ID and password: **sadmin/sadmin**. Once logged as **sadmin**, specific user IDs, passwords and administrative rights may be set.

4. Select **Setup/Instrument** in the task bar. A dialogue box will open:

Figure 2 Instrument Setup. Under the **Instrument** tab: select 9600 or autosense for Baudrate. In COM port, enter COM port as determined in step **2.** In Instrument Type, select instrument currently connected to the PC.

5. Once the Serial Number and Type are shown in the dialogue box, the instrument is connected to the PC.

## Test Definition: defining read and analysis parameters

1. Configure the analysis parameters in Test Definition. Select **Setup>Test Definition** or in the menu bar**.**
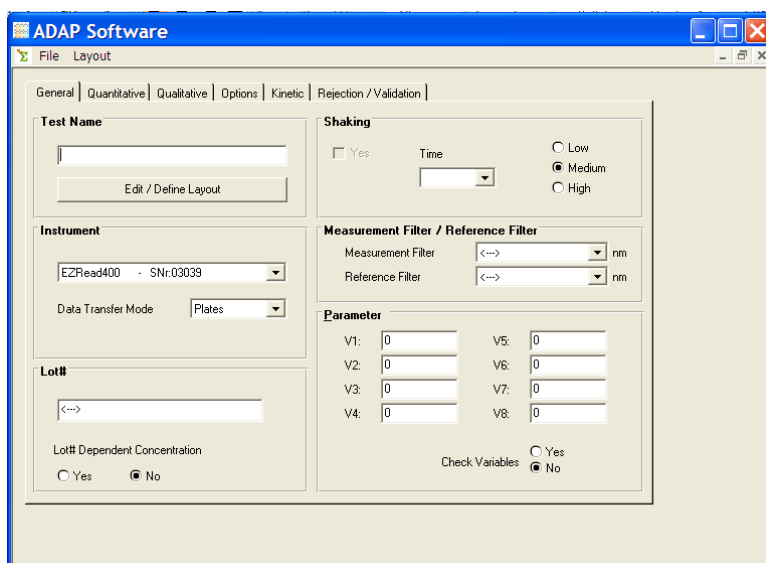
Figure 3 ADAP Test Definition Enter basic measurement details including: method name, and measurement and reference filters. Options include: lot# (if a lot-controlled reagent is used in the assay), parameter (used for inputting calibrating factors), and shaking.

Note: Selecting **Yes** for **Check Variable** prompts the user for an assay lot-specific parameter value before the assay plate is measured.

2. Determine if the samples tested by the assay will be assessed <u>quantitatively</u> or <u>qualitatively</u>. A <u>quantitative</u> assay uses a standard curve to determine concentrations of samples. A <u>qualitative</u> assay uses controls to assess the data and then categorize sample i.e. as "positive" or "negative".

3. Next, configure plate layout by selecting **Edit/Define Layout** below the box for the **Test Name**. Select the position, type and number of the controls before inputting the position of the samples.
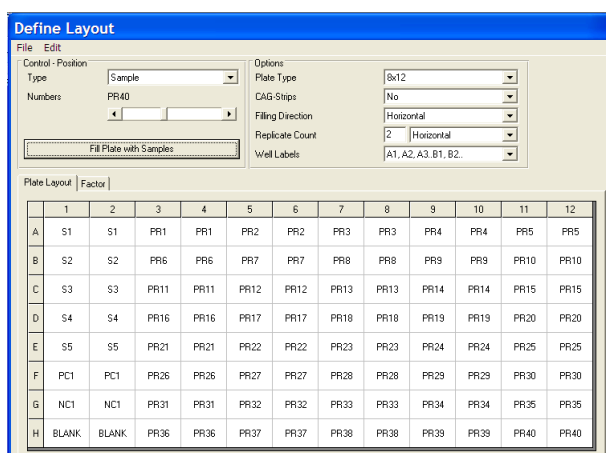
Figure 4 Plate Layout
Select **Type** and **Number** and then use the cursor to highlight the desired location of the well and select enter to place on the plate layout.
**Blank (BL):** will be automatically subtracted from other wells
Qualitative controls: positive control (PC), negative control (NC), quality control (QC) or control (K)
Quantitative controls: standards (S)

- More than one control can be used by increasing the number (found below the **Type** drop down menu) before placing the variable on the plate layout.

- Configure auto fill of samples on the plate layout by selecting options for replicate number and count, and filling direction. Once these parameters have been determined, select **Fill Plate with Samples**. Save the plate layout by selecting **File>End**.

- The factor tab can be used to use a dilution factor (F) configurable to each well.

  Note: F can be entered in transformation formulas and refers to the individual multiplication factor for each well position entered in Factor. F is typically used in quantitative transformation formulas to correct the concentration of samples for their dilution factor; for example, X' = X * F.

4. For a quantitative assay, enter values used for the standard curve with the corresponding response formula. The response formula must refer to the standards defined in the plate layout.
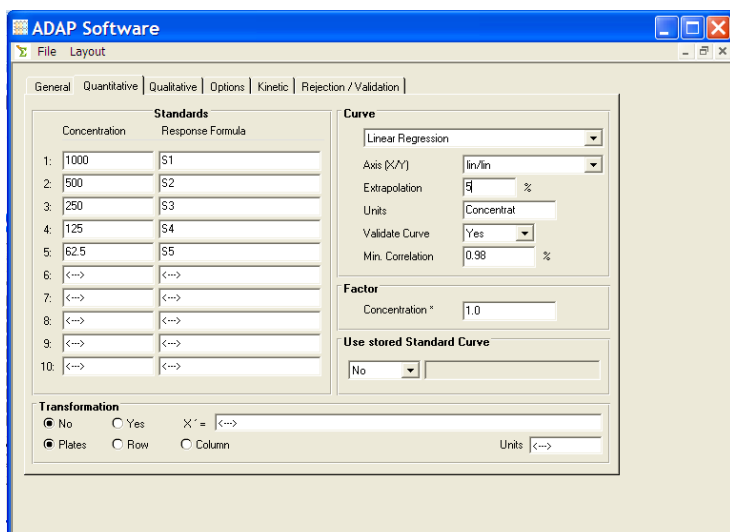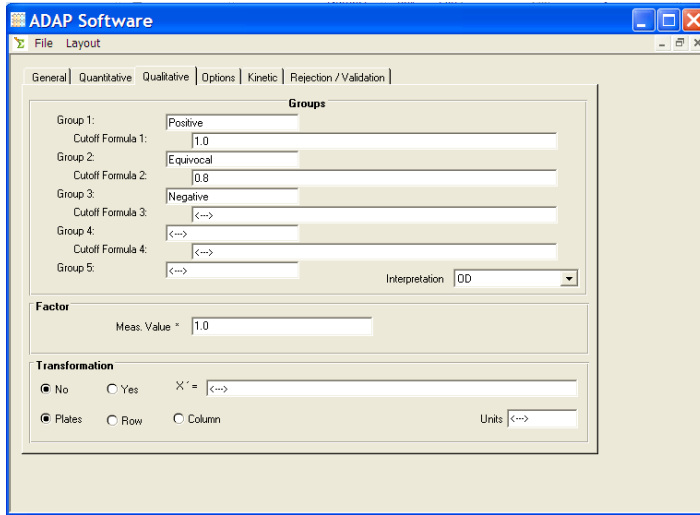

Figure 5 Quantitative Assay In the example to the left, a standard curve has been defined as S1-S5. The concentration is entered under **Concentration** on the left and the standard name is entered for the **Response Formula**. Select the type of curve fitting that will provide the best fit of the data. Other options include**:** percent extrapolation (see table 1 for extrapolation guidelines), choice of linear or log scales for x and y axes, selection of concentration units and minimum correlation (r^2 value) of fit of data to curve for assay verification.

Note: Transformation for quantitative assays is only possible in ADAP Expert.

Table 1 Curve Fitting Models in ADAP

Model	Definition	Restrictions
Point to Point	Interpolation Straight line between each successive data points	No extrapolation 2 points minimum
Linear Regression	Best straight line through a set of data points	3 data point minimum
Cubic Spline	Interpolation of non-linear data	3 data point minimum
4-Parameter	Symmetrical S-curve fit of data	4 data point minimum

5. For a qualitative assay, in **Group 1**: enter in the label that will be applied to the sample if it less than the value(s) defined in **Cut-off Formula 1**. All other samples will be included in Group 2 unless an additional cut-off formula is defined. Thus, two cut-off formulas define 3 groups. Samples included in **Group 1** have values less than **Cut-off Formula 1;** samples included in **Group 2** have values greater than **Cut-off Formula 1** but less than **Cut-off Formula 2**. Samples with values greater than **Cut-off Formula 2** will be include in **Group 3.** Samples can be included in groups based on the raw OD or mathematical transformation.

Figure 6 Qualitative Assay

In the example on the left, samples are categorized as positive or negative by the cut off formula. In the example shown to the left, sample absorbance measurements less than the absorbance of NC1 (negative control 1) + 0.100 will be labeled as "Negative", sample measurements greater than NC1+0.100 will be labeled as "Positive". The basis for interpretation is OD or absorbance measurement although other means of interpretation are possible (table 2).

Additional analysis options include: using a factor, unit labels and transformation calculations. The measurement value must be included in formula and is indicated by "X".

Table 2 Interpretation Options

Interpretation	Definition
OD	Optical Density or raw absorbance data
Transformation	Result from qualitative transformation
Trans (Conc)	Result from quantitative transformation
Concentration	Sample concentration determined from a standard curve

6. The options tab is used to configure calculation of mean values, blank subtraction, printout and control evalution.
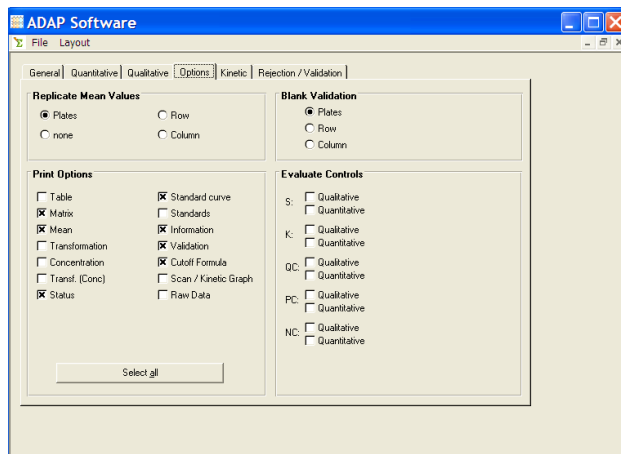
Figure 7 Test Defintion Options

Mean values of replicates can be calculated across the plate or just a row or column that contains the control. Likewise, blanks values can be subtracted from all the wells of the plate or just the row or column containing the blank.

7. Configure replicate rejection and test validation formulas. Formulas programmed in the **Rejection/Validation** tab are used to reject replicates or invalidate tests that do not meet certain conditions.
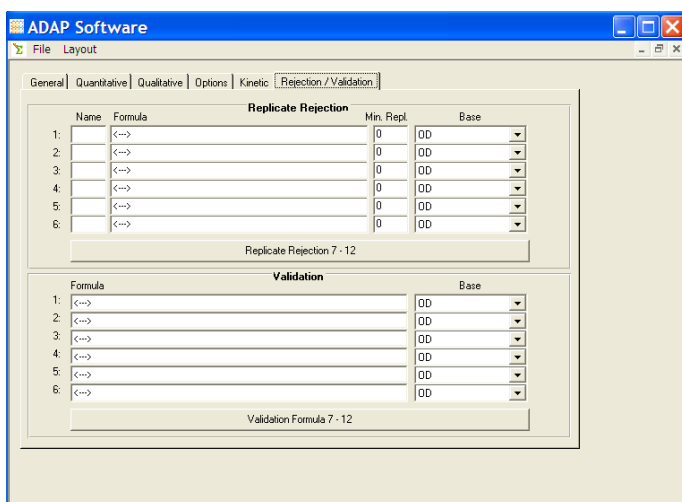
Figure 8 Replicate Rejection and Test Validation
Up to 12 replicate rejection and test validation formulas can be defined. An example of a replicate rejection formula:

> **Name**: PC1
> **Formula**: X>0.700
> **Min Repl**.: 2
> **Base**: OD

This formula will reject any replicate of the control labelled PC1 which has absorbance reading less than 0.700 and the mean of the remaining replicates will be re-calculated. If fewer than 2 replicates remain after the replicates have been rejected, the test is invalid.

Test validation formulas determine the reliability of the test based on the performance of the controls. Test validation formulas are defined similarly to replicate rejection formulas with the exception that if the formula is true, the entire test is rejected. Formulas can include number, controls and mathematical and logical operators listed in Table 3.

Table 3 Logical and Mathematical Operators Used in ADAP

Operator	Definition
AND	True if all conditions are fulfilled
OR	True if one or more of the conditions is fulfilled.
NOT	True if the condition is not fulfilled.
XOR	True if exactly one of the conditions is fulfilled.
ABS	Absolute value
POW	Raises a number to the power of an exponent
SQR	Returns the square root of a number.
L	Returns the natural logarithm of a number
CV	CV% value of replicates
V	Variable 1 to variable 6
F	dilution factor (Rejection formulas only)
X	Actual well value of base (OD, Transformation, or Concentration) during calculation

8. Save test definition using **File/Save**.

9. To measure a plate using the test definition, select **Reading>Single Test** or . Select the desired test definition in the resulting window and OK. A window will prompt you for number of samples. If the numbers of samples are fewer than those defined in the plate layout, then enter in the number and the plate layout will delete the extraneous samples.

    Note: Additional analysis options are available for the Zenyth 200 and Zenyth 340 readers.