

ZYMOPTIQ Technology

User guide

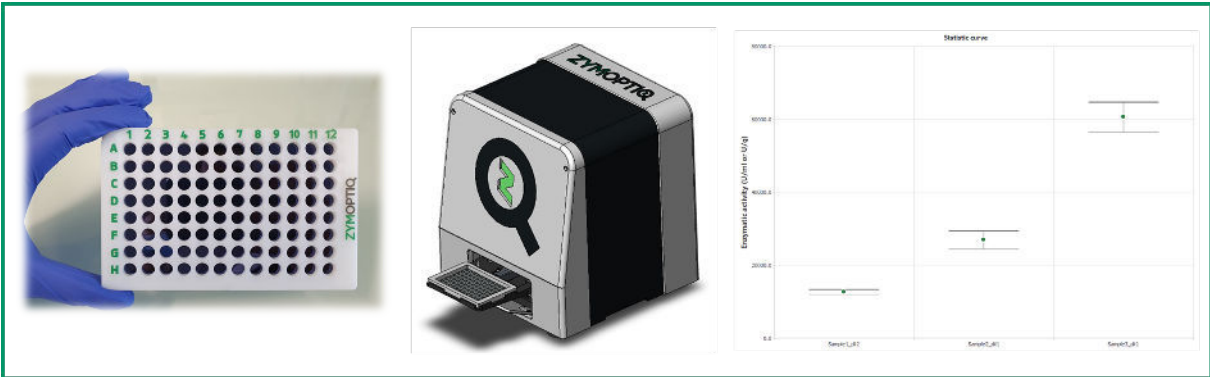


Table of content

1	Preamble	5
1.1	Principle of the method	5
1.2	Samples	5
2	Using Zymplates	5
2.1	Preparation of the incubation	5
2.1.1	Before starting	5
2.1.2	Preparation of the working solutions	5
2.1.3	Preparation of the transfer plate	6
2.2	Starting the incubation	6
2.3	Ending the incubation	6
2.4	Other rinsing solutions	7
3	Preparing to read the Zymplates: Zymocube and Zymosoft	7
3.1	Zymplate and experiment information	8
3.2	Inserting the plaque into the Zymocube	8
4	Filling the plate layout and launching the acquisition	9
4.1	Declaration of the plate layout via "Edit experimental data"	10
4.1.1	"Blank" type wells	10
4.1.2	"Reference" wells	10
4.1.3	"Sample" wells	10
4.1.4	Defining areas	11
4.1.5	Keyboard shortcuts	11
4.2	Saving the plate layout	11
4.3	Declaration of the plate layout via "Select template"	12
4.4	Declaration of the plate layout via "Import experimental data"	12
4.5	Launching the acquisition	13
5	Analysis of results	14
5.1	General	14
5.1.1	Recommended verification sequence	14
5.1.2	Exclusions	14
5.1.3	Units	15
5.1.4	Sorting data in the tables	15
5.1.5	Graphics	15
5.1.6	Plate layout	15
5.2	Verification of the negative controls	15
5.3	Verification of the calibration curve	15
5.4	Verification of the samples	16
5.5	End of data analysis and saving	16
5.6	Files automatically exported by Zymosoft	16
5.6.1	Analysis file in ".zym" format	16
5.6.2	Experiment folder	16
6	Using the Zymosoft in advanced mode	17
7	Automatic exclusions	17
7.1	Negative controls	17
7.2	Calibration points	18
7.3	Samples	18
8	Troubleshooting	19
8.1	Error cases	19
8.2	Error messages	19

1 Preamble

Before using Zymplates and their associated reader: the Zymocube, it is important to read their respective instructions. These documents contain important information about the safety, proper use and conservation of these instruments.

1.1 Principle of the method

The goal is to implement an enzymatic assay to determine the activity of an enzyme solution via the sensors contained in the wells of the Zymplate. A sensor is into contact with the solution containing the enzyme in its buffer. After a defined time, the solution is rinsed out and the Zymplate is dried before being read using the Zymocube. The reading step and data analysis using the dedicated software are also explained in this document.

1.2 Samples

Three types of solutions are required to use Zymplates:

- Negative control: buffer or product without added enzyme, "Blank"
- Dilution range of the enzyme to be assayed, made from a sample of known activity, "Reference"
- Dilution(s) of the sample(s) to be tested, "Sample"

The number of dilutions, dilution factors, number of sampling points as well as the activity range covered by the standard curve should be adapted according to the samples to be assayed and the sensors to be used.

It is recommended to design a plate layout indicating the position of the samples in the Zymplate before performing the experiment. The declaration of the plate layout in the software interface is detailed in the section "Preparing to read Zymplates: Zymocube and Zymosoft".

2 Using Zymplates

2.1 Preparation of the incubation

2.1.1 Before starting

Make sure that the Zymplate has not expired.

Make sure that the consumables, chemicals and glassware needed for the experiment are available (see section "List of consumables, reagents and equipment not supplied" of the Zymplates user guide).

If needed, pre-heat the oven to the desired incubation temperature. Zymplates can be incubated at temperatures ranging from 4 to 70°C.

Prepare the chosen buffer solution. No incompatibility was observed with commonly used buffers (e.g. sodium, acetate, phosphate, citrate ...) at molarities between 0 and 200 mM. Zymplates can be used with buffers at pHs 3.0 to 9.0.

2.1.2 Preparation of the working solutions

The three types of solutions required for the use of Zymplates (negative control, dilution of the reference enzyme and sample(s) to be tested) have to be prepared according to the plate layout.

Notes: Some enzymes may be sensitive to temperature, in such cases, their preparation protocol should be adapted to limit their degradation.

In order to limit the adsorption of enzymes to the vessel and/or to the transfer plate (see next paragraph), we recommend using vessel designed to limit protein adsorption and/or the adding BSA (Bovine Serum Albumin) to a final concentration of 0.5 g/L in the buffer solution.

If the samples to be measured are formulated or if the enzyme is in a complex medium (with substances potentially inhibiting its activity) it is recommended to use this complex medium, without enzyme, as a negative control and as a background solution for the reference enzyme dilution range.

2.1.3 Preparation of the transfer plate

Put the prepared enzymatic solutions in a 96-well plate, according to the chosen plate layout.

2.2 Starting the incubation

Open the packaging and take out the Zymplate.

Using a multi-channel pipette, transfer 80 μ L of the solutions from the transfer plate to the corresponding wells of the Zymplate.



During this step, it is very important to make sure NOT TO touch the bottom of the Zymplate wells. Touching the bottom of the wells could damage the sensor and make it impossible to read the well.

When every solutions have been transferred, seal the plate with an adhesive film to prevent evaporation of the solutions during the incubation. To ensure adhesion of the film, use the provided film-sealing paddle.

Put the Zymplate in an oven / incubator set to the desired temperature. Zymplates are designed to be used at temperatures between 4°C and 70°C.

2.3 Ending the incubation

Take out the plate from the oven, remove the adhesive film and empty the wells by plate inversion over a sink or bin. Wash all wells with distilled water. If rinsing is done with a wash bottle, make sure that the water jet pressure is low, to prevent damage of the sensors. It is also recommended to rinse the plate from bottom to top to prevent possible inter-well contamination. Empty the wells again and repeat the operation a second time.

In some cases, additional rinsing of the wells might be needed, see section "Additional washes".

After rinsing, dry the plate by spinning it for 2 min, wells facing outwards to allow liquid droplets to be expelled. We recommend placing absorbent paper on the Zymplate to collect droplets and avoid damaging the centrifuge.

Before starting the centrifuge, make sure it is balanced: 0.1 g maximum difference using the provided mock Zymplate if needed. If multiple Zymplates were used simultaneously, they can be balanced with each other. The weight of absorbent papers must be taken into account when balancing the plates.

After two minutes of centrifugation, check the wells for residual water. If many droplets are present, centrifuge again. If there are few or no droplets left, let them evaporate at room temperature.

The Zymplate is now ready to be read.

Note: *If the Zymplate is not immediately read, it should be kept in its packaging to be protected from dust and dirt.*

2.4 Other rinsing solutions

If complex solutions have been incubated in the wells of the Zymplate, additional rinses may be necessary to ensure a good reading. The rinsing solutions described below have been tested by Zymoptiq and do not alter the activity results of the samples in most cases. In rare cases, interference has been observed. In these cases, the additional rinsing step should preferably be carried out directly after the first rinse, without intermediate drying of the Zymplate.

- Sodium hydroxide rinsing solution: sodium hydroxide 20 mM dissolved in distilled water. This solution is useful in case the tested product contains many particles, especially if they are of protein nature.
- Tween rinsing solution: Tween 80 from 0.3 to 1% (w/v) dissolved in distilled water. This solution is useful in case the tested product contains fatty contaminants.

Additional rinsing can be used after a problematic reading or be done at the end of incubation, during the first rinsing step. If an additional rinsing is carried out at the end of incubation, it must be preceded by a distilled water washing step. Any additional rinsing should be followed by at least two washes with distilled water (or more if necessary, e.g. in the case of Tween: to get rid of the foam).

3 Preparing to read the Zymplates: Zymocube and Zymosoft



Make sure that the centrifuge is turned off before starting a Zymocube acquisition, especially if these two equipment are placed side by side. Vibrations produced by the centrifuge could interfere with the acquisition of results.

The different steps for using the Zymosoft software (thereafter referred to as: Zymosoft) are described below.

Switch on the Zymocube and wait for about twenty seconds before launching the "ZymoSoft.exe" software. If the software is launched too quickly after turning on the Zymocube, and connections with the computer are not yet established, a pop-up will display an error message. If it happens, close the Zymosoft, wait for a few seconds and restart the software. When the software opens correctly, the homepage will be displayed (**Figure 1**).

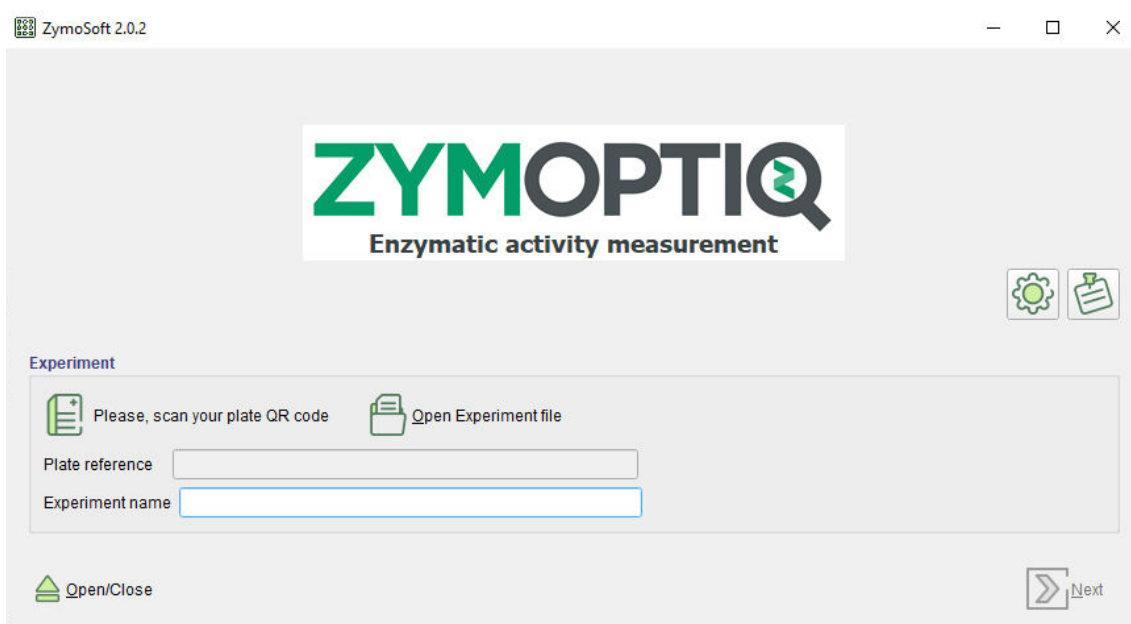


Figure 1 Homepage of the Zymosoft.

3.1 Zymplate and experiment information

First, using the scanner, scan the QR code of the plate to be read by the Zymocube. The cursor should be in the "Experiment name" text bar when scanning the QR code (**Figure 1**). After a beeping sound, the plate reference will be displayed in the top bar and the plate-specific information are available for the analysis.

Write the name of your experiment in the "Experiment name" text bar. This name and the plate reference (provided by the QR code) will be present in the name of the folder containing the results of the acquisition.

Note: The following signs cannot be used in the experience name: [] \ / : * ? " < > |, if they are used, an error message will be displayed.

The Zymplate can now be loaded into the Zymocube. Click on the "Open/Close" button to get access to the drawer.



Before opening the drawer, check that nothing stands in the way!

3.2 Inserting the plaque into the Zymocube

Place the plate on the drawer as shown **Figure 2**: the plate should be put against the lower right corner, the well A1 should be in the upper left corner and the wells should face upwards.

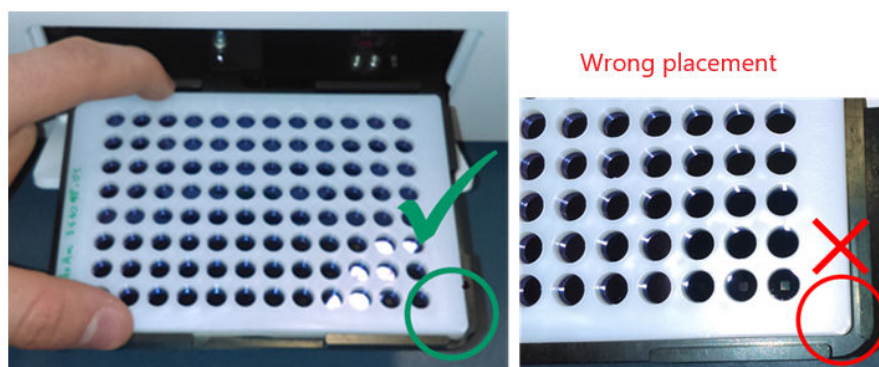


Figure 2 Positioning a Zymplate in the drawer



The plate must lie flat in the drawer before closing it. Incorrect placement of the plate can lead to acquisition problems or even material damage inside the Zymocube.



Risk of pinching when closing the drawer. Do not hold the flap open and remove any tools or fingers from the drawer before closing it.

Click on the "Open/Close" button to close the drawer. Then click on "Next".

4 Filling the plate layout and launching the acquisition

After clicking on "Next", the plate layout page is displayed (**Figure 3**). The plate layout contains information about the contents of the wells of the Zymplate. This can be done either by creating a plate layout via the "Edit experimental data" editing interface, by using a plate layout template via "Select template", or a previously created plate layout (saved in Excel or CSV format) via "Import experimental data".

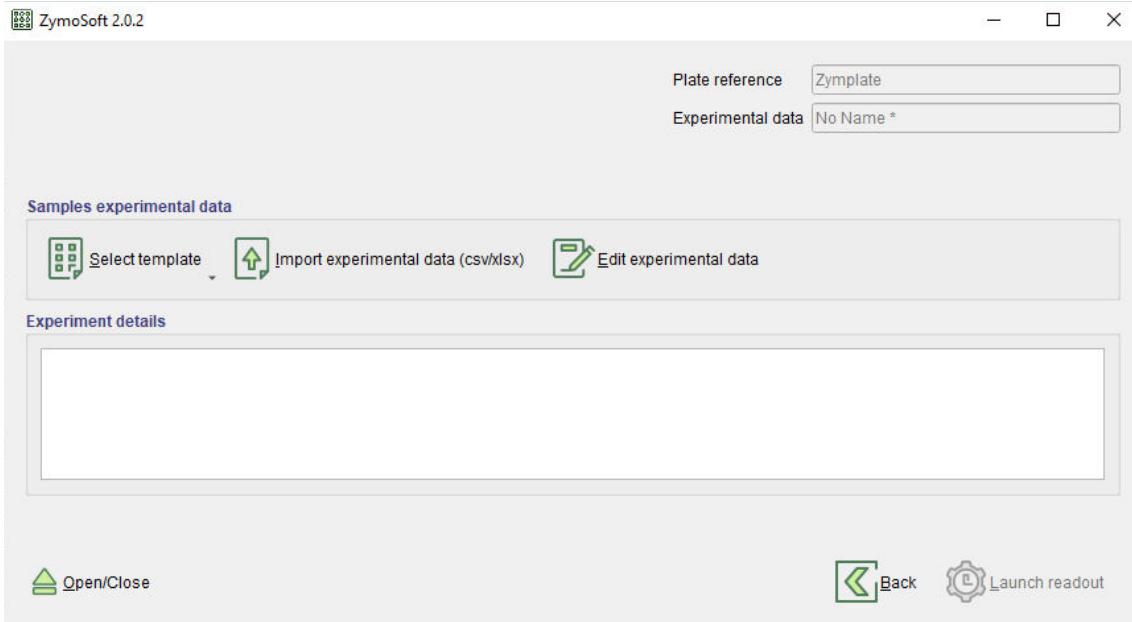


Figure 3 Plate layout homepage

4.1 Declaration of the plate layout via "Edit experimental data"

By clicking on the "Edit experimental data" button, the plate layout editor page opens to an empty plate layout (Figure 4). In the editor, it is possible to set the nature of the wells corresponding to samples deposited in the wells of the Zymplate, their name, activity, dilution factor, ... This information will then be used by the software during data analysis.

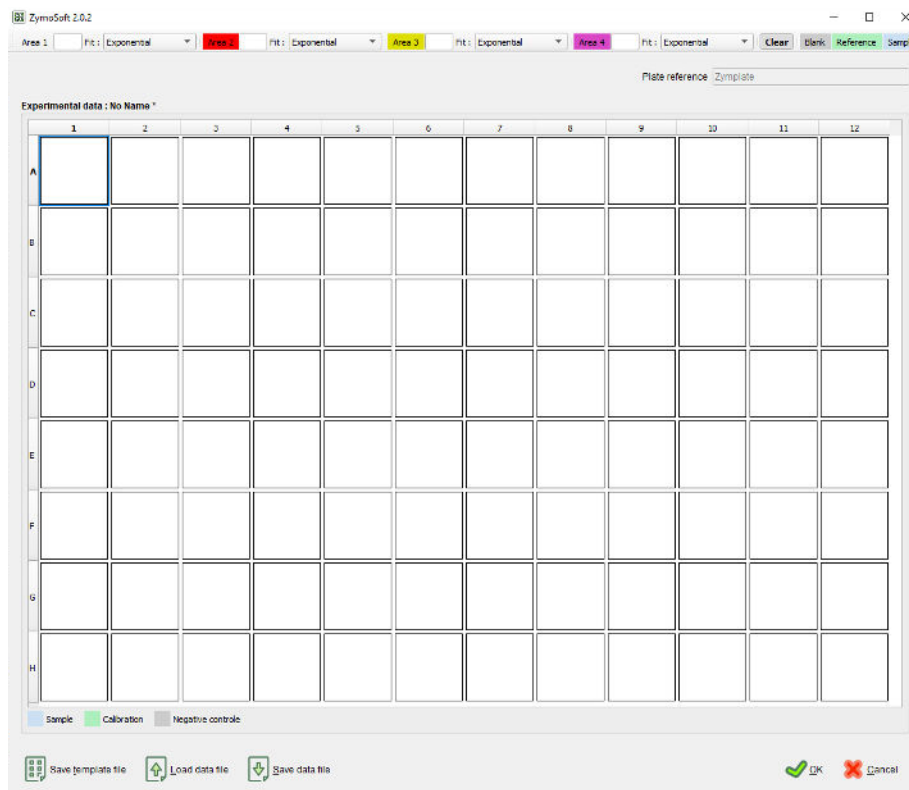


Figure 4 Plate layout editor: starting from an empty plate layout

First, the nature of the wells should be defined according to the nomenclature presented in the "Samples" section. To do this, select one or more wells (a selected well appears with a blue outline)

then click on one of the three buttons at the top right corner of the screen: "Blank" / "Reference" / "Sample". Once the nature of the well is defined, the background of the well is colored (according to the color code present in the interface) and information fields (different depending on the well type chosen) appear.

4.1.1 "Blank" type wells

For negative control wells, there is only one field: the name. All blank wells in the same area (see "Defining zones") must have the same name.

4.1.2 "Reference" wells

For reference wells, two fields are present: one for the name, the other for the enzymatic activity of the solution deposited in the well. Regardless of the name used, wells in the same area with the same activity will be considered as replicas during data analysis.

4.1.3 "Sample" wells

For sample wells, a name field is present and two options for reporting the dilution level are possible. If the "Massic" box is unchecked, only one field: "d=", is present and it should be filled with the total dilution factor applied to the sample. If the "Massic" box is checked, two additional fields appear: "V=" and "m=". These two fields are used to indicate the mass of the sample weighed (in grams) and its volume of resuspension or solubilization (in milliliters). If this information is provided, the field "d=" should correspond to the dilution applied to the samples after this solubilization or resuspension step.

4.1.4 Defining areas

Up to four areas can be defined on a single plate. Their analysis will be carried out independently by the software after the acquisition of the plate.

To assign a well to an area, its type should already be defined, the method is then similar to what was described for the selection of the well type: one or more wells are selected, and assigned to an area by clicking on the "Area" buttons. The outline of the well then appears colored according to the area in which the well is assigned. Area's colors are as follows: colorless for area 1, red for area 2, yellow for area 3 and pink for area 4.

Each area must contain at least one "Blank" position. For each of the areas used, a name must be defined in the field following the "Area" button. It is also possible to choose the type of equation that will be used for fitting the standard curve. The available equations are: exponential, linear and logistic (with 2, 3 or 4 parameters).

Notes: *By default, all wells are assigned to Area 1.*

Wells belonging to the same area do not have to be contiguous.

It is possible to have undeclared wells. The acquisition and analysis of the results will only be done for the declared wells.

Deleting all the information of a well (nature, name, activity/concentration, area) can be done either via the "Clear" button located at the left of the well type definition buttons, or by using the "Delete" key from the keyboard.

4.1.5 Keyboard shortcuts

Most common keyboard shortcuts work in the plate layout editor:

- Multiple selection: Ctrl+Click or by dragging the mouse
- Area selections (rectangular) via Shift+Click
- Copy and paste using keyboard shortcuts Ctrl+C and Ctrl+V

An additional feature with memorization of the order of well selection has been added. It is thus possible to make a multiple selection in a precise order via Ctrl+Click, to copy it with a Ctrl+C, and then to select via Ctrl+Click the wells in which to paste the data of the copied wells keeping the defined order.

4.2 Saving the plate layout

The plate layout is linked with the acquisition of the plate and will, by default, be saved in the file generated at the end of reading and analysis. It is also possible to save it independently via the "Save template file" and "Save data file" buttons.

Using "Save template file" allows the recording of part of the information of the plate layout created: only the types of wells and the areas will be kept. Saving in this format makes the template directly accessible in the software interface.

Using "Save data file" allows the recording of all the data of the plate layout (area, type of well, name, ...) in the form of an Excel or CSV export. Saving in this format is done in on the computer. The recording is independent from the current analysis: modifying the plate layout in the Zymosoft will not result in any modification of the exported file, and reciprocally. This type of file can subsequently be imported and edited in the Zymosoft.

4.3 Declaration of the plate layout via "Select template"

By clicking on the "Select template" button, a drop-down menu allows you to access the plate layout previously saved as templates. Once a template is selected, it opens in the plate layout editor. It contains information about well type, areas, area(s) name(s), and fitting equation type. The template layout must then be filled with the required information according to the procedure described in the section "Declaration of the plate layout via "Edit experimental data"". The plate layout is still fully editable and areas or well types can be modified.

4.4 Declaration of the plate layout via "Import experimental data"

By clicking on the "Import experimental data" button, a navigation window opens to allow you to select on your computer the Excel or CSV file to be used as the plate layout. This file can either come from an earlier backup of a plate layout or be manually created. This type of file contains, in a spreadsheet format (**Figure 5**), the same information as those previously listed in the section "Declaration of the plate layout via "Edit experimental data".

Zone 1	Exponential							
Zone 2	Exponential							
Zone 3	Exponential							
Zone 4	Exponential							
well	zone	type	name	product form	volume (mL)	mass (g)	dilution factor	activity (U/mL)
A1								
A2								
A3								
...								
H12								

Figure 5 Plate layout in a spreadsheet format

The first four lines correspond to the definition of the areas. The first column contains the name of the area (if the cell is empty, then the area is not declared), the second column corresponds to the

fitting equation (if the area is not defined this cell may be empty; by default, the Zymosoft uses "Exponential" type fitting equation).

The rest of the table contains information about each well, using the same format as the one described in the section "Declaration of the plate layout via "Edit experimental data".

- Well: location of the well
- Zone: number of the area to which the well belongs – number from 1 to 4.
- Type: the type of the well. The three defined types are: "calibration", "negative control" and "sample". The types of wells are pre-set in the software, it is therefore imperative to strictly respect this nomenclature for good communication with the software.
The use of an undefined well type will result in the presence of white cells in the plate layout in the software interface and their exclusion from analysis.
- Name: free name
- Product form: in this column two entries are possible: "solution" or "powder". This information impacts the information to be provided in the following columns.
- Volume (mL): this column is taken into account for samples declared as "powder" in the "product form" column. It should contain the volume (in milliliters) in which the sample was extracted or solubilized.
- Mass (g): this column is taken into account for samples declared as "powder" in the "product form" column. It should contain the mass (in grams) of sample extracted or solubilized.
- Dilution factor: this column contains the dilution factor that has been applied to each sample. In the case of a sample declared as "solution", write the total dilution factor. In the case of a sample declared as "powder", write the dilution factor applied to the sample after extraction/solubilization (the dilution coming from the sampling will be calculated from the information contained in the columns "volume" and "mass").
- Activity (U/mL): this column should be filled in only for "calibration" (or "Reference") points. Fill this column with the activities of the solutions of the reference enzyme as put in the wells.

Note: Excel and CSV plate layout files can also be imported via the plate layout editor by clicking on the "Load data file" button.

If you create a plate layout using the Zymosoft interface, you will be asked if you want to save it in Excel or CSV format. If you answer no, the plate layout will not be named and the " Experimental data" field will display "no name". The plate layout data, however, is saved by the Zymosoft linked to the plate acquisition.*

If you modify a plate layout using the Zymosoft, you will be asked if you want to save it in Excel or CSV format. If you answer no, a star will be added at the end of the name of the original plate layout (signaling that the layout has been modified).

4.5 Launching the acquisition

After creating/editing the plate layout and verifying it in the Zymosoft interface, click on the "Ok" button. The software checks that the plate layout meets the following requirements:

- All declared wells (with a defined well type) are named and their information are filled;
- Each area, with at least one declared well, is named;
- Each declared area contains at least one "Blank" well;
- All "Blank" wells within an area have the same name;

If any of these conditions are not met, an error message pops-up, and the user is invited to correct it and validate the modified layout.

The page shown in **Figure 3** is then displayed again and the "Launch readout" button is now active (green icon). Click on this button to start the plate acquisition. You will then be able to hear sounds coming from the Zymocube, indicating the acquisition started. During the acquisition, do not shake, shock or have strongly vibrating equipment close to the Zymocube, as it could impair data acquisition or even damage the hardware.

After a few minutes, the progress bars for data acquisition and processing will progress simultaneously. When the acquisition is completed, the results are automatically displayed in the interface.

Analysis of the results can be done immediately in the software interface or at a later time by reopening the ".zym" file in the Zymosoft. This file is automatically created and saved when clicking on the "End" button.

Clicking on "End" also allows returning to the starting interface to start acquiring a new plate. Click on "Open/Close" to open the drawer and retrieve the plate.

Note: *In the event that a second imaging of the same plate is planned, the plate should be stored in its packaging.*



If several acquisitions in a row are done, the drawer should be opened and closed between each acquisition, to verify the good positioning of the Zymplate and allow the Zymocube to redo its calibration, guarantying good results. This should be done even if the same Zymplate is acquired several times in a row.

5 Analysis of results

The results of an acquisition are available as soon as a plate acquisition is done. Interactive analysis is available in the Zymosoft interface. Saved data can also be reopened from the software homepage, by clicking on "Open Experiment file" and loading the desired ".zym" file.

5.1 General

The starting interface for data analysis is shown in **Figure 6**. Each area declared in the layout is independently treated, and displayed in separate "tabs". Click on the "Area" buttons (top left) to shift from one area to another. All area tabs are organized in the same way, and if multiple areas are present on the plate, the analysis process described below should be done on each area used.

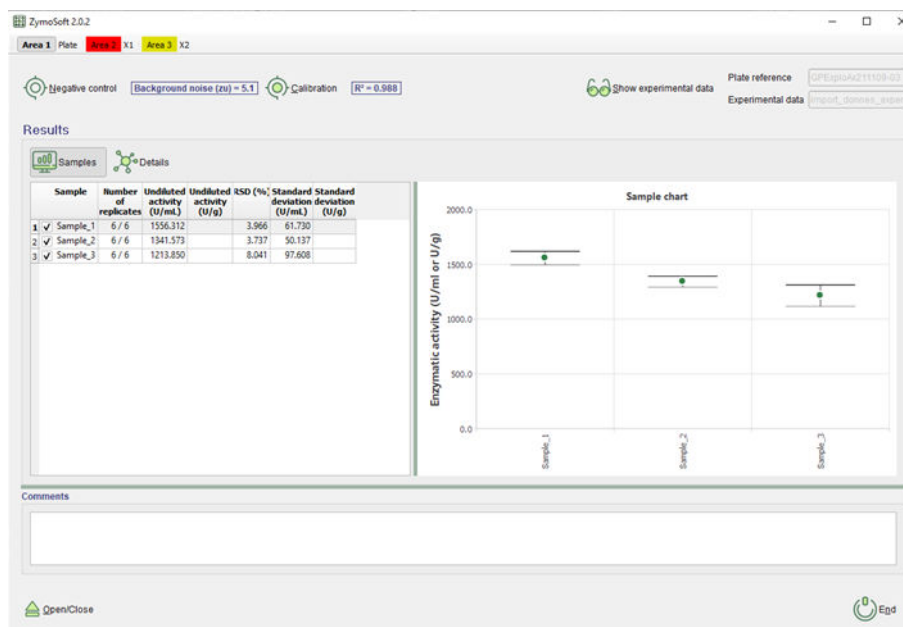


Figure 6 Results interface

5.1.1 Recommended verification sequence

In order to ensure the quality of the results it is recommended to first verify the negative controls, then the calibration curve and finally the samples. Any exclusion/inclusion of a blank well impacts the calculation of the background noise, which in turns impacts the calibration points and their fitting equation which modifies the calculated samples' activities.

5.1.2 Exclusions

For each well, regardless of its type, a "Trouble readout" column indicates through a colored indicator whether the acquisition of the well went ok (green), or not (red). If the indicator is red, the well is automatically excluded. For each well (in the "Details" tab for samples), the exclusion column presents checkboxes to exclude points manually if necessary. The adjacent cell allows you to comment manual exclusions. Automatic exclusions can also be made automatically by the software. In such cases, the reason is then mentioned in the column "Exclusion comment". Some of these exclusions can be canceled by the user, the well is then re-included in the analysis but the automatic exclusion comment remains.

5.1.3 Units

The results of negative controls and calibration curve are displayed in Zymunits (Zu). The results of sample wells are displayed in Zymunits and, if a calibration curve was included, in your U/mL or U/g unit system. If no calibration curve is present, the software displays an error message, and the results are only available in Zymunits.

5.1.4 Sorting data in the tables

All columns in the ZymoSoft's interface can be sorted by alphabetical order or numerical value by clicking on the heading. When this is done in the first sample interface, changing the displaying order of the samples in the table also changes the order in the associated graphic (Figure 6).

5.1.5 Graphics

In all graphics areas from the ZymoSoft, it is possible to zoom in/out on the X axis using the mouse wheel, and to zoom in/out on the Y axis using Shift+wheel. It is also possible to move the visualized area by dragging it with the mouse. A double click allows returning to the initial configuration. Using the right mouse click, it is possible to copy or save (in PNG format) the current view of a graph.

5.1.6 Plate layout

From the homepage of the results interface, it is also possible to view the plate layout that has been used and the information it contains. To do this, click on the "Show experimental data" button at the top right of the window (**Figure 6**). The plate layout is then displayed and all the data and values it contains, which have been used for data analysis, can be viewed. Changing the plate layout is not allowed after acquiring plate data (unless advanced mode has been enabled before initiating acquisition – see section "Using Zymosoft in advanced mode").

5.2 Verification of the negative controls

The Zymunit values of the negative control positions can be accessed by clicking on the "Negative control" icon located at the top left of the window. The exclusion or re-inclusion of points leads to a recalculation of the background noise. The chart on the right side of the panel displays the calibration curve and its fit. If a change is made, it must be saved by clicking on the "OK" button, which also allows you to exit this view. If no modifications were made, or if you do not want to keep the modifications, the "Cancel" button allows you to exit this view without saving changes.

5.3 Verification of the calibration curve

By clicking on the "Calibration" icon you access the page summarizing information about the calibration points (**Figure 7**). The table on the left displays the results of each calibration well. The graph on the right shows the mean and standard deviation for each concentration used, as well as the resulting fit. The equation used for fitting, the values of the parameters and the coefficient of determination, R^2 , are also presented. At the end of the verification, click on "OK" (to save changes) or "Cancel" (to exit without saving changes) to return to the results homepage.

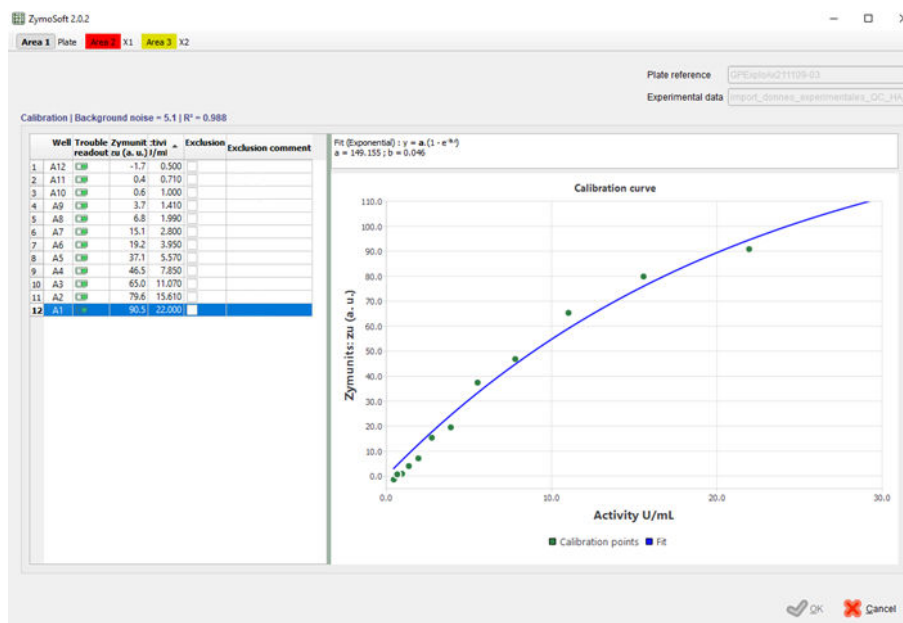


Figure 7 Calibration curve interface

5.4 Verification of the samples

The sample homepage view shows sample results aggregated by names. For each group of samples, the number of points kept, the corresponding undiluted activity value obtained, its standard deviation and its Relative Standard Deviation (RSD) are presented in the table. In this table, check boxes allow you to select the samples to be displayed in the graph. Changing their order in the table also changes the order in which they are displayed in the graph located on the right side of the window.

By clicking on the "Details" button, you can access a table containing the results obtained for each well. This table lists the results in Zymunits, diluted activity and undiluted activity. It also allows you to view automatic exclusions made by the software, and add or remove exclusions manually.

5.5 End of data analysis and saving

When data analysis is completed, click on the "End" button in the bottom right corner of the window. The results are then automatically saved and exported. A file in the format ".zym", readable by the Zymosoft and containing all plate layout and analysis data, is generated. A folder containing various exports as Excel files and PNG images is generated as well.

5.6 Files automatically exported by Zymosoft

5.6.1 Analysis file in ".zym" format

This file, readable by the Zymosoft software, contains all the data acquired and the treatments that have been applied to it. It can be reopened in the Zymosoft software (via the "Open experiment file" button on the homepage) to allow for data analysis and/or to allow you to generate new visuals.

In case modifications are made through the Zymosoft's interface (e.g. new exclusions, ...), clicking on "End" enables you to save the new data analysis and will overwrite previously saved files.

5.6.2 Experiment folder

In the folder associated to the experiment (same name and plate reference as the ".zym" file), you will find "WellResults" files in CSV and Excel formats. The "WellResults" file contains the raw data in Zymunits, the calibration curve fitting information as well as the activity results (U/mL or U/g) of the samples. In case no calibration curve was present, or if the fitting equation did not converge on the data points of the calibration, "WellZymUnitResults" files in CSV and Excel formats, containing only Zymunits, are generated.

For all Excel results files, if the plate layout contained multiple areas, the results of each of the areas are presented in different tabs. Image files, in PNG format, are also automatically exported. For each area of the plate layout, a "Calibration_curve" file and a "Sample_chart" file are generated, respectively containing the graphical representations of the calibration curve and fit, and samples results (as visualized in the Zymosoft).

6 Using the Zymosoft in advanced mode

On the homepage of the Zymosoft (**Figure 1**), a wheel icon allows you to activate the advanced mode by entering a password (**Figure 8**). In addition to what was previously described, activating the advanced mode allows the modification of the plate layout after data acquisition.

Notes: *the advanced mode must be activated before acquiring the plate.*

When reopening a ".zym" file in the Zymosoft, you should also enable advanced mode before opening the ".zym" to enable changes to the plate layout.

To modify the plate layout, in the data analysis interface, click on the "Edit experimental data" button located top right of the window (this button replaces the "Show experimental data" from the non-advanced mode). The software then displays the plate layout in the editor and all wells can be modified: area, well type and field content.

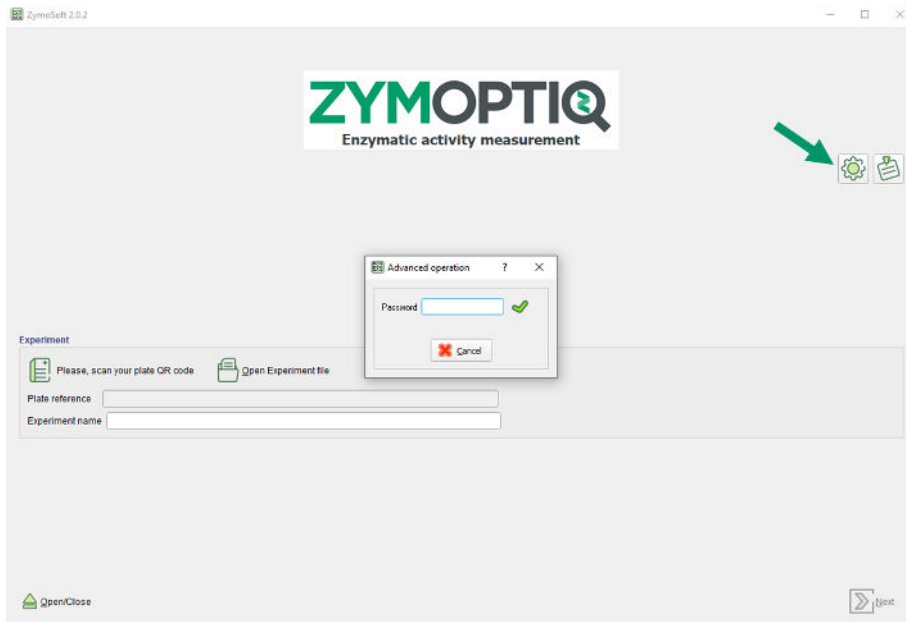


Figure 8 Enabling advanced mode

7 Automatic exclusions

Some positions can be automatically excluded during analysis by the software. The underlying reasons for exclusions and their associated comments are presented in the following paragraphs depending on the nature of the wells concerned.

7.1 Negative controls

For each sensor type, limits in Zu are defined for negative control wells. A negative control position that does not meet the defined criteria will be excluded.

If more than a third of the negative control positions are excluded, then the analysis will not proceed and an error message will be displayed in the software interface. One common case is insufficient drying of the plate, in such cases a message prompting. After checking the dryness of your plate, the correct declaration in the plate layout of the "Blank" positions, or other issues, restart the acquisition.

7.2 Calibration points

Calibration points must have values between 2.5 and 90 Zu. Wells that do not meet this criterion are automatically excluded, and the comment "Not valid" is written in the "Exclusion comment" column.

If the calibration curve was replicated on the plate, the exclusion of a point at a given activity, automatically leads to the exclusion of the other point(s) of the same activity level and the comment "Not valid" is displayed.

In case of an issue during the acquisition (red indicator in the "Trouble readout" column), the concerned well is excluded, no Zymunits are displayed and the comment "Not valid" is written. In this case, the cells are shaded which means that the well cannot be manually reincluded by the user during analysis.

7.3 Samples

The Zymunit values of the samples should be within the range covered by the calibration curve. In order to take into account, the fact that replicas are often used, the limits have been set to 0.8 x lowest calibration point and 1.2 x highest calibration point for the lower and upper limits,

respectively. Samples that do not meet this criterion are automatically excluded, their Zymunit value is displayed but their activity is not calculated.

- If the detected Zymunits are below the lower limit, the comment "Too diluted" is displayed.
- If the detected Zymunits are too high, but below 90 Zu, the comment "No activity value" is displayed.
- If the detected Zymunits exceed 90 Zu, the well is automatically excluded and the comment "Too concentrated" is displayed.
- If the detected Zymunits are lower than the "Background noise", the well is automatically excluded and the comment "Not valid" is displayed.

In case of an issue during the acquisition (red indicator in the "Trouble readout" column), the concerned well is excluded, no Zymunits are displayed and the comment "Not valid" is displayed. In this case, the cells are shaded which means that the well cannot be manually reincluded by the user during analysis.

If no calibration points were included or declared in the plate layout, all sample points appear as excluded. However, their Zymunits are visible in the "Details" tab with the exclusion comment "No reference" (if they were not excluded for one of the reasons listed above).

8 Troubleshooting

8.1 Error cases

Description	Solution
The software does not start or the Zymocube does not respond.	The bandwidth between the software and computer might be overloaded. This could happen if the software is run twice or if some background tasks are in progress. Complete the current tasks and restart the Zymocube and then the Zymosoft.
The USB cable was unplugged during use.	The Zymocube freezes. Reconnect the cable, restart the Zymocube and then the Zymosoft. Restart the acquisition.
The computer shuts down during acquisition.	The Zymocube freezes. Restart the Zymocube and then the Zymosoft. Restart the acquisition.
The data acquired do not correspond to the experience analyzed.	Check that the QR code of the Zymplate and the plate layout entered in the software are correct and then restart the acquisition.

8.2 Error messages

Context	Error message	Explanation/Solution
Launching the software	"The Zymocube controller has been interrupted!?"	The Zymocube is not turned on or has not had time to connect to the computer before the launch of the software.
Advanced mode	"The password is incorrect!"	The password is incorrect.
Label Scan	"The V2.0 QR code format is not valid!"	The QR code of the label is not in the right format.
	"The volume count ... in the QR code format is not valid! »	The QR code of the label is not in the right format.
	"The QR code format is not valid!"	The QR code of the label is not in the right format.
	"The QR code version is not valid!"	The QR code of the label is not in the right format.

Context	Error message	Explanation/Solution
Import of the plate layout	"The row and/or column count is not valid!"	Edit Excel or CSV file.
	"Unable to read the values!"	Edit Excel or CSV file.
Plate Layout Editor	"The area ... is not defined! »	The area ... is not defined.
	"The volume value is not valid!"	Volume value is invalid.
	"The mass value is not valid!"	The mass value is not valid.
	"The dilution value is not valid!"	The dilution value is not valid.
	"The activity value is not valid!"	Activity value is invalid.
	"The area index ... is not valid! »	The index value of an area is not valid.
	"Unable to write the template file!"	The plate layout template was not saved.
	"The name of well at the position ... is not defined. »	The well ... has no name.
	"The dilution factor at the position ... must be greater than 0.0. »	The sample well must have a dilution factor greater than 0.0.
	"The volume at the position ... must be greater than 0.0. »	The volume defined for the well ... must be greater than 0.0.
	"The mass at the position ... must be greater than 0.0. »	The mass defined at the well ... must be greater than 0.0.
	"The sample... can not be massic and liquid! »	A sample cannot be defined as liquid and massic.
	"The area ... does not contain blank well. »	The area ... contains no negative control wells.
	"The blanks in the area ... should have the same name. »	All negative control wells within the area ... must have the same name.
	"The activity at the position ... must be greater than 0.0. »	The calibration well ... must have a declared activity greater than 0.0.
	"The name of area ... is not defined."	The name of the area ... is not defined.
	"The name of the template is not defined!"	The template name is not defined.
"The experience name contains invalid characters ([] \ / : * ? " < >)!"	The experience name cannot contain the following characters: [] \ / : * ? " < >	

Context	Error message	Explanation/Solution
Launch of the acquisition	"Unable to launch the controller process: ...!"	Restart the computer, Zymocube and Zymosoft.
	"Unable to launch the controller process (no temp. directory)!"	Incorrect installation of the software. Contact Zymoptiq.
	"Unable to launch the controller process (no config .ini file)!"	Incorrect installation of the software. Contact Zymoptiq.
	"The Interf parameters file ... do not exist! »	Incorrect installation of the software. Contact Zymoptiq.
	"The Interf parameters file is not defined for the plate...!"	Incorrect installation of the software. Contact Zymoptiq.
	"Unexpected controller error ... »	Hardware problem. Contact Zymoptiq.
	"Unable to create the data directory!"	Incorrect installation of the software. Contact Zymoptiq.
	"Unable to build the experiment file!"	Incorrect installation of the software. Contact Zymoptiq.
Opening ". zym »	"The file does not contain a valid Zymocube experiment!"	The selected file is not in the right format.
Viewing results	"Trouble readout"	Problem during the well acquisition. Rinse, re-dry and reimage.
	"Unable to exploit the area"	Negative controls of an area do not allow the results of the wells to be exploited.
	"The blank wells are not valid!"	Negative control wells are not valid.
	"The reference wells are not valid!"	Calibration wells are not valid.
Validation of results	"Please check write permissions"	Verify that no Excel or CSV files that may interfere with the current analysis are opened.