byonoy

Instructions for Use
Absorbance 96 App

App version 1.5

$C \in$

Manufacturer:	Byonoy GmbH	00 49 40 5379 866 00
	Schützenstraße 21	service@byonoy.com
	22761 Hamburg, Germany	www.byonoy.com

TABLE OF CONTENTS

1   General information

The Absorbance 96 App is to be used only in combination with the Absorbance 96 product family. The Absorbance 96 App is not a stand-alone product. Please also refer to the separate operating instructions for the Absorbance 96 reader.

The Absorbance 96 App is only compatible with the Absorbance 96 reader. The safeties, precautions and validations included in the Absorbance 96 App have only been tested for the Absorbance 96 and shall not be used for any other purpose on any other device.

In this manual, particularly important remarks are labeled as followed:

Warning:

⚠️   **This is a warning that indicates an immediately or potentially dangerous situation, which, if not avoided, will result in death or serious injury.**

Caution:

✋   **Caution indicates a potentially hazardous situation, which, if not avoided, could result in minor or moderate injury.**

Information:

ⓘ   **This is a piece of information indicating certain properties that must be observed.**

About this manual

Important: Follow the instructions for use

This user manual may only be used according to its intended purpose. It may not be reproduced, changed, or translated into another language without the prior written consent of Byonoy GmbH.

This document is subject to technical changes and updates.

ⓘ   **Ensure that the manual is always accessible and is read and understood by all persons operating the instrument.**

ⓘ   **The latest version of this manual is available via the Absorbance 96 App.**

2   App installation procedure and updates

The Absorbance 96 App is compatible with Microsoft Windows (Windows 7 or above) and Mac OS (Mojave 10.14. or above).

To install the Absorbance 96 App, visit the following webpage: s.byonoy.com/abs96. Download the latest version of the App for your operating system and follow the steps in the Setup Wizard to complete the installation.

Modern operating systems offer plug and play functionality. Older operating systems may require manual installation of the drivers. The drivers for Windows are available at s.byonoy.com/abs96.

> ⓘ **To install the proper drivers and software on the computer, the user must have administrator rights or obtain such permissions.**

Uninstalling the software

The Absorbance 96 App can be uninstalled using the computer's standard uninstall procedure.

Updates

To check for new updates, click the "Update" button in the lower left corner. If an update is available, this will be shown in a bar at the top of the screen.

3 Absorbance 96 App overview

The Absorbance 96 App is used to operate the Absorbance 96 reader and processes the results. The main window appears automatically after the app is started and the reader is connected.
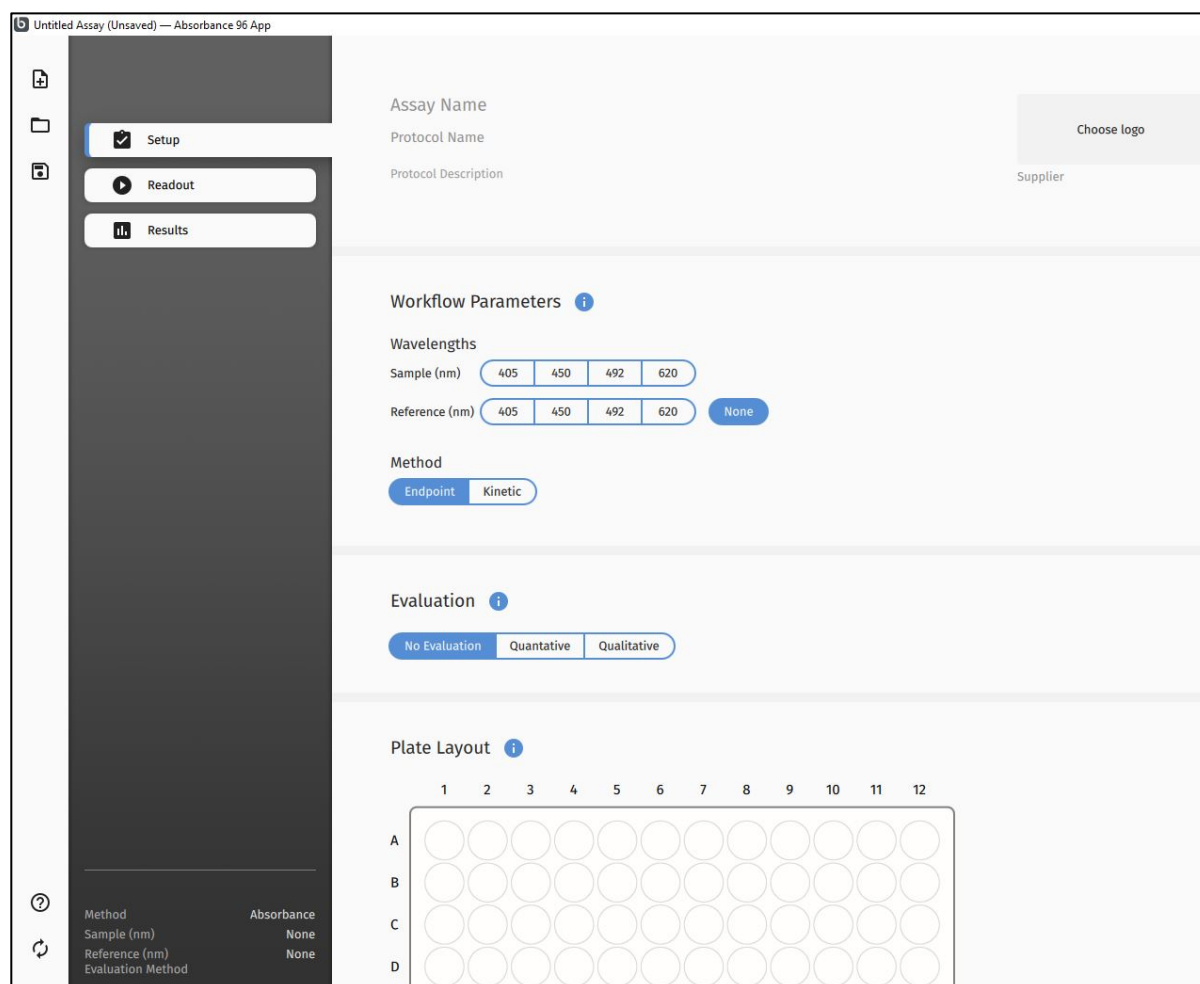

Figure 1. Main window.

Icons in the leftmost menu bar (from top to bottom):

1. New: Start creating a protocol and/or assay.
2. Open: Open a previously saved protocol or assay.
3. Save As: Save a current protocol or assay.
4. Manual: Redirection for downloading the user manual.
5. Update: Update the app.

File Types

The following file types are used by the Absorbance 96 App:

Table 1. File Types in the Absorbance 96 App

Description	Extension
Protocol	.byop
Assay	.byoa
Export	.csv/.pdf

The protocol file type .byop allows to save settings and to load predefined protocols for repeat assays.

The assay file type .byoa is used to save completed assays. In addition to the settings, the results of the assay are also saved.

4   Operation

4.1   Set up an assay and create a protocol

Select "New" from the menu bar. Your experiment conditions can now be defined.

(i)   **The Absorbance 96 App can connect via SiLA2 to enable remote execution of commands including loading a protocol, performing a measurement, and exporting the results as a .csv file.**

4.1.1   Workflow Parameters

The workflow parameters can be expanded via the gray, dotted button for view options. This button expands a menu of options that can be added to the master view.
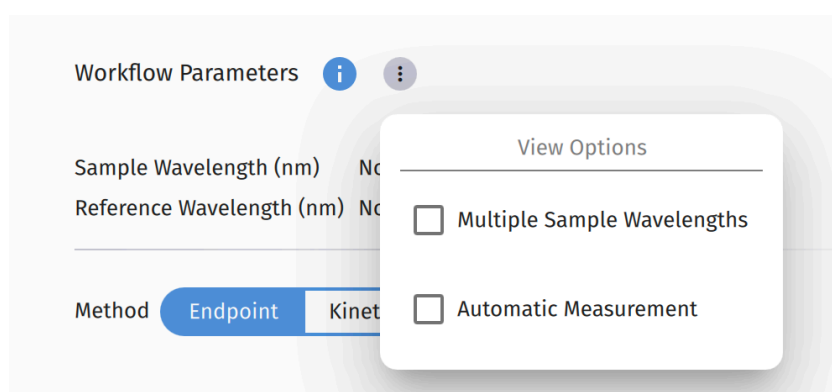

Figure 2. Expanded View Options Menu

1. **Multiple Sample Wavelengths**

When selected, multiple sample wavelengths will add the option to measure multiple wavelengths sequentially.

2. **Automatic Measurement**

When selected, the automatic measurement will add the option to perform an automatic measurement and auto-export of the results. This enables workflows where one protocol is being used across multiple plates.

3. **Wavelengths**

Under "Wavelengths", a signal and reference wavelength (both in nanometers) may be chosen.

The signal wavelength is the wavelength at which the principal measurement will be run.

The reference wavelength is an additional wavelength at which the device will measure and whose results will be subtracted from the values measured at the signal wavelength. If no reference wavelength is desired, click the "None" button beside the reference wavelength.

4. Method

Select your readout method, either endpoint or kinetic.

ⓘ **The kinetic readout method is not available for every reader type.**

To perform kinetic measurements and avoid misinterpretation of the results, observe the following instructions:

ⓘ **Ensure constant environmental conditions, i. e. constant ambient temperature and humidity.**

ⓘ **Acclimatize the connected reader and microtiter plate to the ambient temperature for at least 1 hour.**

ⓘ **A signal drift of about 1 mOD per hour might be observed.**

4.1.2 Kinetic Rapid Mode

Upon selecting kinetic mode, you can choose "Enable Rapid Measurement". In this mode, the hardware is no longer performing the additional verification step and results can therefore be delivered in about 3 sec. intervals.

ⓘ **Enabling Rapid Measurement may affect the accuracy, linearity and reproducibility of the measurement.**

4.1.3 Evaluation

The evaluation section allows one to choose which evaluative method will be used for the readout.

The view options menu can be expanded to show the following additional features.

1. Limit Extrapolation

Select Limit Extrapolation to limit the extrapolation of calculated concentrations to the maximum known standard concentration multiplied by the extrapolation factor.

2. Predefined standard curve

In a quantitative assay, a predefined standard curve can be used to determine the concentrations of a new set of samples with identical experiment parameters. After selecting the

checkbox "Use predefined standard curve", you may enter the curve parameters manually or load a standard curve from a previous assay.

> ⓘ **After selecting the checkbox "Use predefined standard curve", you cannot assign any new standards.**

> ⓘ **In a quantitative assay, the standard curve is a function of various assay parameters, such as the temperature, timing, or age of the assay kit. Before using this feature, it is advisable to validate your assay for compatibility.**

> ⓘ **As the above-mentioned experiment conditions are subject to change, it is recommended to use a new standard curve when performing a new assay.**

No Evaluation

No evaluation will be used. Only the OD values will be shown.

Quantitative

Upon selection of the quantitative evaluation method, a drop-down menu will appear. Here, a fitting method can be chosen. The selected fitting method will be used for the standard curve and the data analysis.

Qualitative

Upon selecting the qualitative evaluation, an editor will appear that allows for the definition of the parameters for the qualitative evaluation.

Figure 2. Editor for qualitative evaluation

Terms for the Editor:

- Input Formula: the input to be used for the evaluation.
- Limit Formula: definition of the evaluation boundaries. The limit can be entered as a constant value or as a formula.
- Positive: the designation for values above the limit
- Negative: the designation for values below the limit

To add an intermediate area between the positive and negative fields, hover with the cursor over a given field, whereupon a "+" symbol will appear. Click to add the intermediate field.

To define your evaluation, klick on the "fx" field under "Input Formula". The Formula Editor will open and you can enter the desired formula.
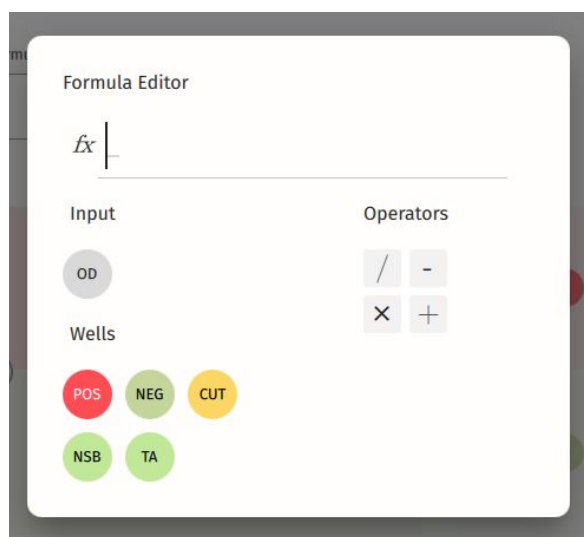

Figure 3. Formula Editor

Terms for Formula Editor:

- fx: the entry field for the input formula
- Input: the selected input for the formula
- OD: input the measured values in OD from each well
- Operators: mathematical operators to build the desired formula
- Wells: well-specific controls
- POS: input the mean of the positive controls
- NEG: input the mean of the negative controls
- CUT: input the mean of the calibrator or cut-off control

All input formulae must contain OD. If no formula is desired, the input will be solely OD.

Example 1

A simple formula may take the form:

Cut-Off Value = mean negative controls + 0.2,

To enter this formula, set the following:
Input Formula = OD
Limit Formula = NEG + 0.2

Figure 4. Simple formula for cut-off

Example 2

A more complex formula, for example, for the representation of values as a percentage of the negative control, or the percent inhibition, the entries for the qualification would be as follows:

Inhibition = (1-(ODsample/ODnegative control))*100

The figure below shows the input for the percent inhibition calculation, where samples greater than or equal to 30 % would be designated positive.
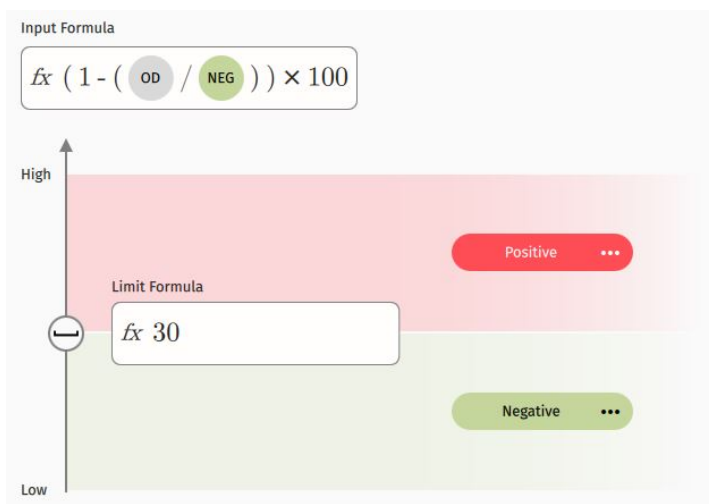
Figure 5. Formula for calculation of percent inhibition

Note: click the bracket symbol to switch the direction of the limit inclusion.

ⓘ **The examples shown in this manual do not represent actual data, rather function to clarify software features, and may not be referenced for experimental methods or procedure.**

4.1.4   Plate Layout

In the plate layout section, samples, standards, blanks, and controls can be defined. Select the type from the options below the plate layout; click and drag to define. Duplication patterns allow the user to define multiple replicates for samples and standards.

ⓘ **The format of the plate layout and the corresponding buttons are defined by the evaluation method. Quantitative and qualitative evaluations have different fields and buttons.**

ⓘ **When replicates are defined, the mean value is used for the calculations.**

In the plate layout for a quantitative assay, a field is provided where the concentrations of the standards and the samples can be entered. The unit can be entered manually or chosen from the dropdown menu. Using the dilution factor, diluted samples can be used, and the software will calculate the undiluted concentration.

B0 control

B0 is a specific denomination of zero standard controls in competitive ELISAs. If B0 is chosen, its value is used to reference the relative binding in a sample or standard to the maximum possible binding. This ratio is referred to as the percentage of binding %B0.

Custom well labels

To accommodate user-defined assay parameters, custom well labels are available. A well can be given a designation and color of choice. Wells with custom labels can also be used in formulas.

Create a protocol

Once the parameters of a given experiment have been set, these settings may be saved as a protocol. All parameters, such as wavelength and plate layout, will be saved.

4.1.5 Assay Validation

To provide checks on various assay parameters, a Smart Validation is performed, and custom validation conditions can also be defined.

Smart Validation checks the well assignment on the microplate, the microplate orientation, the %CV of replicates, and checks the monotonicity of the standards.

Custom validation allows many custom validation conditions to be set and evaluated. Any conditions that are set will be automatically calculated by the software and displayed in the results section.

4.2 Readout of the assay

Once all settings have been completed, the read out can be started. The app will automatically guide the user through the readout process.

4.3 Results

After a successful measurement, the results section will open automatically.

(i) **Except for the controls themselves, all data shown in the results section is corrected data, meaning the blank, NSB, or S0 values have already been subtracted. The raw values of blanks, NSB or S0 are used in the assay validation. All other calculations are performed using the corrected data.**

(i) **Expand the View Options Menu to select between %CV Absorbance and %CV Concentration.**

The results are displayed in different views:

Plate view

Provides an overview of the different OD values in their respective wells. Undefined wells are not shown. Click on a given well to see the OD value, label, and, if applicable, concentration.

Graph view

A graphical representation of the data will be shown. The graphical view differs depending on the evaluation.

Quantitative evaluation

If defined, the standard curve is generated based on the fitting method chosen. By choosing the right fitting method, the distance between the curve and each point is minimized. Using the standard curve, the concentration of each sample is calculated. The equation below the graph reflects the fitting method applied and offers further data.

Qualitative evaluation

The samples and controls will be shown on an intuitive plot arranged according to their designation, measured values, and relation to the limit formula.

Assay Validation

The results of both the Smart Validation and the custom validation are displayed. If all criteria of the Smart Validation are valid, they will appear green with a pass marking. If the criteria are not met, a yellow warning will be displayed.

> (i) **A warning does not indicate a failed assay, but rather indicates additional checks may be required.**

If custom validation conditions were set, these will be evaluated and marked either green for pass, or red for fail.

Tabular view

Provides an overview of the various samples, wells, OD values, and calculations.

Table 2. Overview of terms, tabular view section

Parameter	Description
ID	The assigned ID of a given standard
Well	The assigned well
Absorbance (OD)	The absorbance reading in OD units
Mean Absorbance (OD)	The mean OD value of replicate wells
%CV	The coefficient of variation between replicates, measured in OD.

Standard Concentration*	The entered concentration values for the standards
Concentration*	The calculated concentration values
Result**	Pos/Neg/Intermediate result of a qualitative experiment
Input Value**	The calculated value from the input formula

(* Only appears for quantitative evaluations, **Only appears for qualitative evaluations)

Export

Export the results as a .pdf or .csv file.

Automatic plate detection

The microplate must be removed from the device after completing the measurement. If the device detects that a microplate is present in the slot, a warning will appear instructing the user to remove the microplate from the slot.