

# **Product Manual**

# Absorbance 96 plate reader

Catalog #: ENZ-INS-A96

Manufactured by Byonoy GmbH

Device Version 2.0 (2021-01-18) Software Version 1.2 (2021-01-18)

For the latest product information, including support documentation, visit us online:

www.enzolifesciences.com

Technical Support (US): 800-942-0430 Technical Support (EU): +41 61 926 8989

Customer Service (US): <u>info-usa@enzolifesciences.com</u> Customer Service (EU): <u>info-eu@enzolifesciences.com</u>

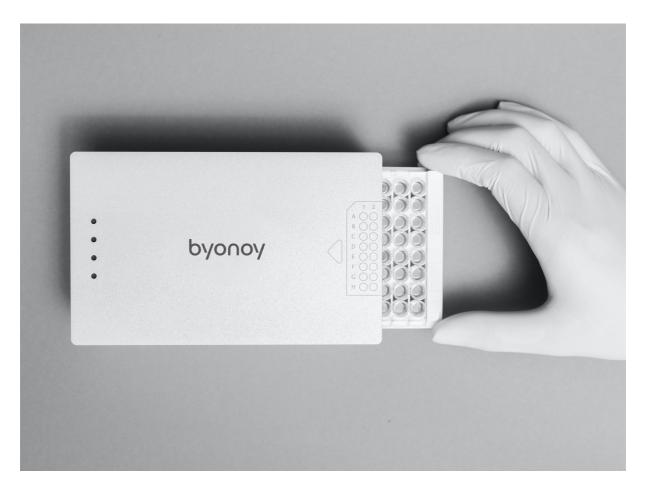
FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



# Instructions for Use

# Absorbance 96

Valid for REF Number ABS MH A 01/ABS MH D 01





Instructions for Use Absorbance 96 Version 2.0 (18/01/2021) ©Copyright 2021 by Byonoy GmbH. All rights reserved.

Manufacturer:

Byonoy GmbH Bernstorffstr. 118 22767 Hamburg, Germany 00 49 40 5379 866 00 service@byonoy.com www.byonoy.com

#### Dear customer,

We are delighted that you have chosen the Absorbance 96. To take full advantage of the instrument's performance and to enjoy your instrument for many years, please read these instructions for use carefully before installation and commissioning. Operate the instrument in accordance with these instructions. The operating-safety and function of the instrument can only be guaranteed if both the general safety regulations and accident prevention regulations of the legislator as well as the safety instructions in this manual are observed. We accept no liability for any damage resulting from improper use or incorrect operation.

(i)

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

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

This document is subject to technical changes and updates.

# TABLE OF CONTENTS

1	General information	1
2	Overview of the Absorbance 96	3
	Scope of application Measurement method	3
	Absorbance 96 System  Consumables	
3	Preparing the product for use	6
	Unpacking, storage, and transportSupplied materials	6
4	Operation	8
	App installation procedure and updates  Computer connection  Assay readout	8
5	Signals and troubleshooting	10
5.1 5.2	Signal lights  Troubleshooting	
6	Maintenance and cleaning	12
6.3	Maintenance  Cleaning  Technical support  Repairs	13 15
7	Technical specifications	16
8	Guarantee	18
9	EC-Conformity Declaration	19

## 1 General information

## Important: Follow the instructions for use

Any commissioning or handling of the instrument requires precise knowledge and notice of this manual. The device is intended for the described use only.

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.

#### Liability for function and damage

Liability for the function of the device shall, in any case, pass to the owner or operator if the device is improperly maintained, repaired, or modified by persons who do not belong to the authorized service personnel, or if it is handled in a way that does not comply with its intended use. The service and operation of the product must be in accordance with this manual. Byonoy shall not be liable for damages resulting from non-observance of the information above. Warranty and liability conditions of the terms of sale and delivery of Byonoy are not extended by the information above.

# Warning



An incorrect working environment can lead to a reduction in service life, damage to the device, or measurement errors. Please note the explanations and warnings in chapter 3.3.



To avoid measurement errors, it is essential to ensure that the instrument is connected properly and that the experiments are carried out correctly. Please note the explanations and warnings in chapter 3.



Incorrect cleaning of the instrument can reduce its service life and can cause damage to the instrument. Please note the explanations and warnings in chapter 6.2.

# Information



Follow all safety instructions on the device and in the attached documents.



Follow all general precautions that apply to electrical instruments.

# Disposal of used electrical and electronic equipment



The symbol on the product or its packaging indicates that this product is not to be treated as normal household waste. It must be disposed of at a collection point for the recycling of electrical and electronic equipment. By contributing to the correct disposal of this product, you protect the environment and the health of your fellow human beings. Recycling helps to reduce the consumption of raw materials. For further information on how to recycle this product, please contact your local authority or municipal waste disposal centres.

## Working with biological and harmful material

The Absorbance 96 is not to be used for the measurement of biohazardous substances.

Always observe the manufacturer's hazard information pertaining to the substances to be measured.

The instrument does not produce any toxic or harmful gases or substances. During the measurement, make sure that there are no toxic or harmful substances in the microtiter plate.

# 2 Overview of the Absorbance 96

# 2.1 Scope of application

The Absorbance 96 is an optical laboratory instrument for measuring the absorbance (optical density) of biological or non-biological samples in ANSI/SBS-standard 96-well microtiter plates in accordance with the specifications described in the user manual. The Absorbance 96 is intended for research and other non-in-vitro-diagnostic analyses only. It is to be operated by trained laboratory personnel and is intended for professional use.

#### 2.2 Measurement method

The Absorbance 96 is designed to carry out sensitive absorbance measurements. It measures the optical density (OD) of samples at defined wavelengths.

# **Absorption**

Absorption refers to the amount of light absorbed by a medium. Absorption reduces transmission. Transmission is the ratio of incident to transmitted light. Accordingly, the degree of transmission is calculated as follows

$$T = (I/I_0)$$

where I is transmitted light and I<sub>0</sub> is incident light.

## **Optical Density**

Optical density is a measure of the attenuation of light radiation after it has passed through a medium. Optical density is the logarithmic quantity that describes the reciprocal of the transmittance T:

$$OD = log(l_0/l)$$

where I is the transmitted light and Io is incident light.

Optical density is the absorbance of the sample plus other attenuating effects such as scattered light due to turbidity. It is therefore necessary to avoid scattered light in order to measure absorbance correctly.

If no other attenuating effects are present, absorbance = optical density.

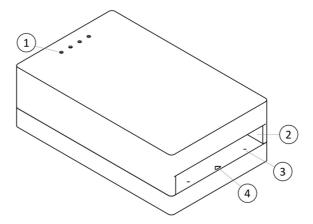
#### 2.3 Absorbance 96 System

#### Absorbance 96 Reader

The Absorbance 96 reader is an absorbance-based measuring device, i. e. a measuring instrument, with which the absorbance values of a 96-well microtiter plate can be read, recorded and provided for further processing. The Absorbance 96 is a solid-state microplate reader and has 96 detection units, allowing measurement without a scanning mechanism.

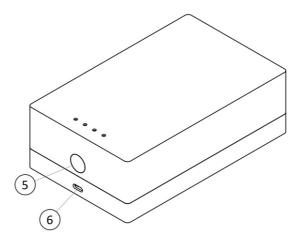
In the upper part of the measuring instrument, there are up to 4 LED light sources with different wavelengths and up to 4 interference filters. The four signal lights on the top of the reader show which measuring channel is being used for the current measurement.

The microtiter plate is inserted manually into the slot of the device. There are two raised locking-edges at the bottom of the slot, as well as an arrow for visual inspection. The arrow becomes wholly visible only after complete insertion of the microtiter plate into the slot.



**Figure 1.** Visual representation of the product from the front, top. 1. Signal lights. 2. Slot for microtiter plate. 3. Raised locking-edges. 4. Arrow.

On the back of the Absorbance 96 there is a Micro-USB port for connecting the instrument to a computer via the included Micro-USB cable. Furthermore, there is an eject button for ejecting the microtiter plate out of the slot.



**Figure 2.** Visual representation of the product from behind, top. 5. Eject button. 6. Micro-USB port.

## Power consumption

The Absorbance 96 is an analytical instrument with very low power consumption. It is operated by a Micro-USB cable and the total power consumption is generally less than 2.5 watts.

#### Absorbance 96 App

The reader is controlled via the Absorbance 96 App. The operation of the app is described in-detail in a separate user manual.

## 2.4 Consumables

With the Absorbance 96, ANSI/SBS Standard 96-well microtiter plates can be used. Only certain microplate types can be used with the Absorbance 96 (see chapter 7).

When using microtiter plates, always check the specifications of the microtiter plate manufacturer. Not all microtiter plates of a particular manufacturer are the same in design, materials, or configuration. The temperature stability within the microtiter plate can depend on the type of microtiter plate used.

# 3 Preparing the product for use

# 3.1 Unpacking, storage, and transport

## Unpacking

Remove the packaging material and carefully place the device on a firm and level surface. Check the device for external damage and check the materials supplied (see chapter 3.2).

The device is packed in a specially designed cardboard box. Keep the packaging material. If the device must be returned for repair, the original packaging material must be used.

Storage and protection during the intervals of normal use



Protect the device from moisture and dust during prolonged storage.



Do not store the device next to heat sources and protect it from direct sunlight.

The temperature for storage should be within the recommended temperature range (see chapter 7).

## Transportation

Before transportation, unplug the instrument and ensure there is no microtiter plate inside the device. Depending on the transport distance, use the original packaging material. Make sure that the new location meets the requirements described in chapter 3.3.



To avoid spillage and contamination, make sure that there is no microtiter plate inside the instrument before transport.



Always transport the product well-packaged to avoid damage in transit.

# 3.2 Supplied materials

When unpacking the device, please check that the following components are present:

- Absorbance 96
- Micro-USB cable
- Hex key
- Microfiber cloth
- USB Stick

# 3.3 Working environment

Due to its small size, the Absorbance 96 can be operated very well in various working environments.

However, the following warnings must be observed. Ignoring them may lead to measurement errors and a reduction in the expected lifetime or damage to the device:



The device should stand on a level surface, free from dust and vibrations.



Do not operate the device near heat sources or under direct sunlight.



The ambient temperature and the humidity should be within the recommended range (see chapter 7).



Place the instrument in a horizontal position secured against falling.

# 4 Operation

Before commissioning the Absorbance 96, you should carefully read and understand the entire manual to familiarize yourself with the system.

The Absorbance 96 is controlled via the Absorbance 96 App, including the analysis of measurement data. The functions of the app are detailed in a separate user manual.

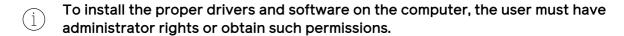
#### 4.1 App installation procedure and updates

The app is compatible with Windows und MacOS operating systems (see chapter 7).

To install the Absorbance 96 App, insert the USB stick into the computer and follow the steps below:

- Locate and select the USB drive in the document browser.
- Open the installation file either for Windows or macOS.
- 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 software drivers. The drivers for Windows are available on the USB stick.

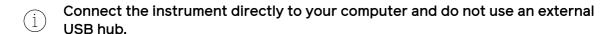


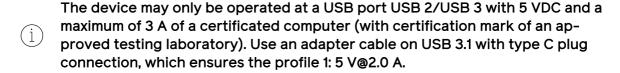
## 4.2 Computer connection

The Absorbance 96 requires a connection to the computer for power supply, starting the experiment, and data transfer. Insert the Micro-USB cable into the Absorbance 96 and the USB port of the computer. The Absorbance 96 starts automatically when connected to a computer via the Micro-USB cable and the software automatically establishes a connection.



Always use the included Micro-USB cable for the connection to your computer.





When the Absorbance 96 is switched on, the signal lights on the top of the instrument light up. Each time the instrument is switched on, an internal self-test is performed to ensure there are no malfunctions.

If no connection between the instrument and the computer is established, install the driver for the USB connection. You can find it in the supplied USB Stick.

# 4.3 Assay readout

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

In order to guarantee a correct measurement result, please pay attention to the indications in the Absorbance 96 App und heed the following warnings:



When inserting the microtiter plate, pay attention to its alignment.



Make sure that the microtiter plate was inserted in the slot completely. In that case, the arrow at the bottom of the slot is visible completely.



The bottom of the microtiter plate must be dry before it is inserted into the instrument. If the microtiter plate is wet at the bottom, dry it with a paper towel or similar.



Shocks of the Absorbance 96 or the microtiter plate can cause an unwanted signal. Therefore, neither the Absorbance 96 nor the microtiter plate should be touched during measurement.



Note that the system depends on a clean and dust-free environment. Therefore, do not wear powdered safety gloves.



Ensure that no direct sunlight or other strong light sources shine into the slot of the Absorbance 96.



Insert the plate cautiously to avoid spills.

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

The microtiter plate may now be removed from the Absorbance 96. To do this, push the eject button on the rear side of the device.

# 5 Signals and troubleshooting

# 5.1 Signal lights

Table 1. Explanation of the signal lights

Activity of the signal light	Meaning
All signal lights flash simultaneously	Error, software shows error message
No signal lights light up	Device is off Device is waiting for input Device is defective
All signal lights light up briefly in sequence	Self-test after connection to power
Signal light of the respective measuring channel flashes/illuminates continuously	Initialization/measurement in progress

# 5.2 Troubleshooting

If there is an error, all four signal lights flash simultaneously. In this case, check the software to see which error is present.

 Table 2. Error messages and solutions

Type of error	Cause	Solution
	There was a microtiter plate in the slot during initialization.	Remove the plate.
Initialization failed.	The inside of the slot is dirty.	Clean the inside of the slot according to the cleaning instructions in chapter 6.2.
	Irrecoverable hardware error.	Contact the service department.
Interference due to ambient light that is too bright.	Too much ambient light is entering the slot.	Make sure the instrument is not exposed to too much ambient light, e. g. direct sunlight, during the measurement process.
Temperature out of specification.	The ambient temperature was too high or too low.	Make sure the ambient temperature is within the specifications.
	Defective USB port.	Use a different USB port on the computer or a different computer.
USB Power Supply Defective (< 450mA or < 4V).	Using a USB hub.	Connect the instrument directly to the computer or use another USB hub.
	Defective Micro-USB cable.	Contact the service department.

Irrecoverable hard-	Irrecoverable hardware	Contact the service department.
ware error.	error.	Contact the service department.

# 6 Maintenance and cleaning

#### 6.1 Maintenance

The Absorbance 96 is maintenance-free. Each time the instrument is switched on, an internal self-test is carried out to ensure there are no malfunctions.

There are no parts within the Absorbance 96 that can be serviced by the customer. It is only necessary to ensure that the device is kept clean.

The accuracy, linearity and reproducibility of the device can be checked with a reference plate. Byonoy recommends the reference plate from Hellma GmbH (item code 666-R013), for which the following steps are described.

# Accuracy

- 1. Measure the plate at the desired wavelength. Ensure that there is no dust or other contamination on the reference plate.
- 2. Compare results taking the measurement uncertainty and specifications of the reader into account and use the following formula:

```
OD_{RP} - MU - AS \le OD_{RES} \le OD_{RP} + MU + AS
```

OD<sub>RP</sub>: Optical density of the reference plate at the desired wavelength. This value is found on the calibration certificate from the manufacturer of the reference plate.

MU: Measurement uncertainty of the reference plate. This value is found on the calibration certificate from the manufacturer of the reference plate.

AS: Accuracy specification of the reader at measured optical density. This value is shown in chapter 7.

OD<sub>RES</sub>: The optical density measured by the reader at the desired wavelength.

# Example:

OD<sub>RP</sub>: 1.554

MU: ± 0.0079

AS:  $\leq 1\% + 0.010 \text{ OD from } 0.0-2.0 \text{ OD}$ 

**ODRES:** 1.573

- (1)  $1.554 0.0079 (1.554*0.001 + 0.010) \le OD_{RES} \le 1.554 + 0.0079 + (1.554*0.001 + 0.010)$
- (2) 1.52056 ≤ OD<sub>RES</sub> ≤ 1.58744

The measured optical density of the reader lies within the calculated range.

- 3. Rotate the reference plate by 180 degrees and repeat steps 1 and 2.
- 4. If all Results (ODRES) are within the range, the accuracy of the device has been verified.

# Linearity

With the Hellma reference plate, you can check the accuracy at 6 different OD points. If the accuracy for all these points has been verified, the linearity of the reader is also verified.

# Reproducibility

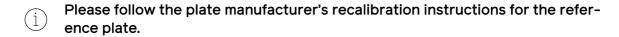
- 1. Measure the plate at the desired wavelength for at least 3 times. Ensure that there is no dust or other contamination on the reference plate.
- 2. Calculate the standard deviation OD<sub>SD</sub> for each well.
- 3. Compare the measured standard deviation and specifications of the reader using the following formula:

OD<sub>SD</sub> ≤ LS

OD<sub>SD</sub>: Calculated standard deviation for each well

LS: Linearity specification of the reader at measured optical density. This value is found in chapter 7.

In case that the results of accuracy, linearity or reproducibility are outside the range, please contact the service department.



## 6.2 Cleaning

Remove the Micro-USB cable from the instrument before cleaning.



Make sure before cleaning, that there is no microtiter plate in the instrument.

# Cleaning the housing

The surfaces of the housing should be cleaned regularly. For this purpose, you can use e. g. a cloth, or a sponge lightly wetted in water. For heavier soiling, clean the surface of the housing with a mild soap solution diluted with water or glass cleaner and then wipe with a lightly moistened cloth or sponge to remove any residue. Do not use scouring agents.

# Cleaning the slot

To clean the slot of the Absorbance 96 open the four screws on the bottom of the instrument (see Figure 3) using the Hex key provided.

Do not loosen any screws that are not explicitly marked in the manual, as this can lead to a malfunction and may void the instrument's warranty.

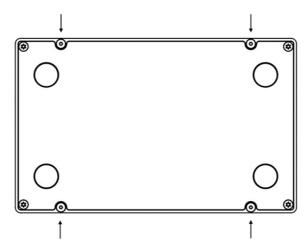


Figure 3. Bottom side of the instrument

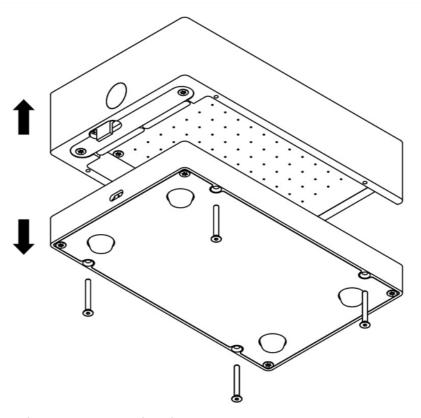


Figure 4. Pull the instrument apart into its two components

Carefully pull apart the top and the bottom of the instrument to separate the plug connection on the back of the instrument.

To avoid damaging the rigid plug connection between the top and bottom of the instrument, grasp the instrument at the back during the pull movement and avoid any tilting movement.

Use a dry microfiber cloth or a bellows to carefully remove dust or dirt from the surface. If this is not enough, the microfiber cloth can be slightly soaked with 80% ethanol to carefully remove dust or contamination from the surface.

Do not allow water or other liquids to get inside the instrument. If this happens, return the device to the manufacturer for inspection.

# Warnings for cleaning

When cleaning, pay attention to the following warnings. Failure to observe these warnings may result in damage to the instrument. This can lead to a reduction of the service life or in measurement errors:



Always avoid spraying liquid directly onto the surfaces of the Absorbance 96. This is especially important for the inside of the slot, where there are optical elements that are very sensitive and essential for the functioning of the instrument.



Never clean the inside of the slot with sharp or abrasive scourges, and do not use aggressive solvents or corrosive agents.



Biological hazard: Always wear gloves during cleaning operations that may involve contact with biological or generally hazardous materials or liquids.

# 6.3 Technical support

In case of problems with the instrument, please contact the manufacturer's service department or your local representative.



Biological hazard: It is your responsibility to decontaminate the instrument and all accessories before servicing and before returning the instrument or accessories to the manufacturer.

For decontamination of the Absorbance 96, follow the government guidelines for inactivation of organisms used in biological laboratories.

## 6.4 Repairs

Repairs on the device may only be carried out by the manufacturer. Please contact the service department. The product warranty is voided if the device is modified by unauthorized persons, or different parts are installed.

# 7 Technical specifications

Table 3. Performance and technical data

Parameter	Value
Type of product	Absorbance reader
Product name	Absorbance 96
Service life	10 years at an average use of 4h/day
Housing material	Aluminium
Place for use	Laboratory
Degree of contamination	2
Temperature for storage/measurement	5-40 °C
Temperature (Transport)	-10-50 °C
Relative tolerated humidity (storage/measurement)	Max. 70 %
Measurement method	Absorbance
Measuring technique	Endpoint and Kinetic
Microplate types	96-well, flat bottom
Light source	Up to 4 LEDs
Filters	Up to 4
Detection	96 Photodiodes
Measurement range	0-4.0 OD
Resolution	0.001 OD
Accuracy*	405 nm: ≤ 1.5 % + 0.010 OD from 0.0-2.0 OD ≤ 3 % + 0.010 OD from 2.0-3.0 OD ≥ 450 nm: ≤ 1 % + 0.010 OD from 0.0-2.0 OD ≤ 1.5 % + 0.010 OD from 2.0-3.0 OD
Reproducibility**	≤ 0.5 % + 0.005 OD from 0.0-2.0 OD ≤ 1 % + 0.010 OD from 2.0-3.0 OD
Linearity***	405 nm: ≤ 1.5 % from 0,0-2.0 OD ≤ 3 % from 2.0-3.0 OD ≥ 450 nm: ≤ 1 % from 0,0-2.0 OD ≤ 1.5 % from 2.0-3.0 OD
Connection to computer	USB 2/USB 3 with 5 VDC and max. 3 A
Dimensions	55 x 96 x 154 mm
Power supply	5 VDC

Nominal value/Characteristic of the fuse	1 A/very fast-acting
Power input	2.5 W
Weight	900 g
System requirements (App)	Microsoft Windows: Windows 7 or above Mac OS: High Sierra 10.13 or above

<sup>\*</sup>Accuracy is the maximum deviation between the determined value and the true value.

<sup>\*\*</sup>Reproducibility is the maximum deviation between the determined values when the measurement is repeated directly.

<sup>\*\*\*</sup>Linearity is the maximum deviation between the true and the determined increase of the value.

# 8 Guarantee

The regular warranty period of the Absorbance 96 is 24 months. If a defect manifests itself in your device during the warranty period, please contact the service department directly.

The instrument may only be operated in technically perfect condition. In the event of defects that could endanger employees or third parties, the device may only be used again after it has been repaired by the manufacturer.

This warranty does not cover damage caused by improper use or external mechanical influences, transport damage, or unauthorized intervention in the device by unauthorized persons.

# EG-Konformitätserklärung EC-Conformity Declaration



# Byonoy GmbH - Bernstorffstraße 118 - 22767 Hamburg - Germany

Das bezeichnete Produkt entspricht den einschlägigen grundlegenden Anforderungen der aufgeführten EG-Richtlinien und Normen. Bei einer nicht mit uns abgestimmten Änderung des Produktes oder einer nicht bestimmungsgemäßen Anwendung verliert diese Erklärung ihre Gültigkeit.

The Product named below fulfills the relevant fundamental requirements of the EC directives and standards listed. In the case of unauthorized modifications to the product or an unintended use this declaration becomes invalid.

Produkttyp Product type

# Absorptionsmessgerät

Typenbezeichnung Type designation

## Absorbance 96

Einschlägige EG-Richtlinien/Normen Relevant EC directives/standards

# 2011/65/EU; 2014/30/EU;

DIN EN ISO 14971:2013-04; DIN EN 62366-1:2017-07; DIN EN 62304:2016-10; DIN EN 61010-1:2011-07; DIN EN 61010-2-101:2017-10; DIN EN 61326-1:2013-07; DIN EN 61326-2-6:2013-07

Hamburg, den 08.08.2019

Dr. Sebastian Metz

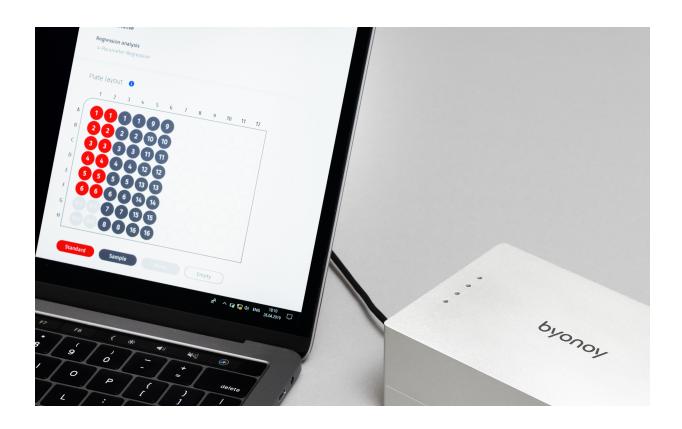
Dr. Yousef Nazirizadeh



# Instructions for Use

# Absorbance 96 App

Version 1.2





Instructions for Use Absorbance 96 App Version 1.0 (18/01/2021) ©Copyright 2021 by Byonoy GmbH. All rights reserved.

Manufacturer:

Byonoy GmbH Bernstorffstr. 118 22767 Hamburg, Germany 00 49 40 5379 866 00 service@byonoy.com www.byonoy.com

# TABLE OF CONTENTS

1	General information	1
2	App installation procedure and updates	2
3	Absorbance 96 App overview	3
4	Operation	5
4.2	Set up an assay and create a protocolReadout of the assay	8
5	EC-Conformity Declaration	9

## 1 General information

The Absorbance 96 App is to be used only in combination with the Absorbance 96 reader. 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 in 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.
- $\widehat{\phantom{a}}$  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 (High Sierra 10.13. or above).

To install the Absorbance 96 App, insert the USB stick into the computer and follow the steps below:

- Locate and select the USB drive in the document browser.
- Open the installation file, either for Windows or macOS.
- 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 software drivers. The drivers for Windows are available on the USB stick.



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 process the results. After the app is started and the reader is connected, the main window appears automatically.

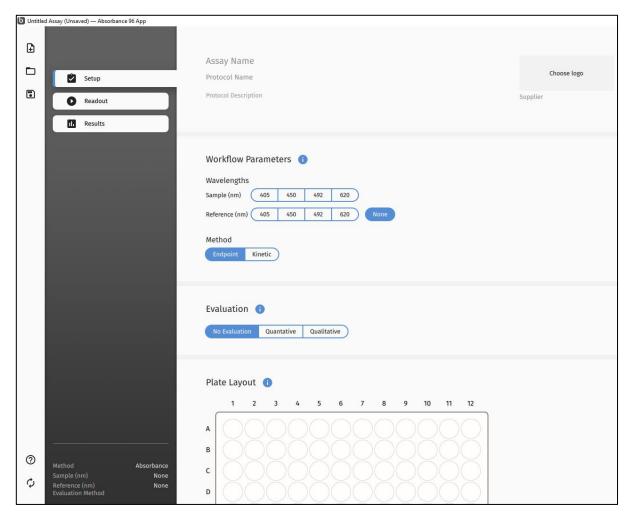


Figure 1. Main window.

# 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: Open the app 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.

#### **Workflow Parameters**

#### 1. 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.

#### 2. Readout Method

Select your readout method, either Endpoint or Kinetic.

ig(i) 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.
- ig(i) A signal drift of about 1 mOD per hour might be observed.

#### 3. Evaluation

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

## 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.

#### 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.

#### Qualitative

Upon selection of qualitative evaluation, a formula for the cut-off definition will appear.



Figure 2. Formula for cut-off definition.

#### Terms for cut-off definition

The terms for cut-off definition from left to right according to Figure 2:

- POS = the mean of the OD values of the positive control wells.
- NEG = the mean of the OD value of the negative control wells.
- CUT = the mean of the OD value of the cut-off control wells.
- 0 OD = zero. User-defined value may be entered.
- Offset = an assay dependent factor provided by the user.
- Range = definition of a range as a percentage value.

The formula setup for the cut-off definition covers multiple formula types. These are listed below.

- Cut-Off Value = mean negative controls +(-) k
- Cut-Off Value = mean positive controls + k
- Cut-Off Value = mean cut-off controls + k
- Cut-Off Range = [mean control + k] +(-) x %

k is an experimentally determined factor normally defined by the laboratory professional or by the kit manufacturer.

#### Example:

To replace the minus function, the user must simply enter a negative value. For example, if the cut-off is

Cut-off = Mean Negative Controls - 0.2,

this must be entered as follows:

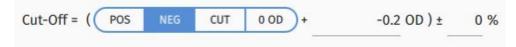


Figure 3. Cut-off calculation with subtraction

In case the Cut-Off is known or provided by the kit manufacturer, and therefore does not need to be calculated, select 0 OD and enter the value into the middle field:



Figure 4. Entering a known cut-off value

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.

## 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.
- ig(i) 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.

#### 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.

# 4.2 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. 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 representation of the data will be shown on the graph. The standard curve is dependent 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.

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

Table 2. Overview of terms, tabular view section

Description
The assigned ID of a given standard
The assigned well
The absorbance reading in OD units
The coefficient of variation between replicates
The entered concentration values for the standards
The calculated concentration values
The mean OD value of replicate wells
Pos/Neg/Intermediate result of a qualita- tive experiment

(\*Only appears for qualitative evaluations)

#### **Export**

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

# EG-Konformitätserklärung EC-Conformity Declaration



# Byonoy GmbH - Bernstorffstraße 118 - 22767 Hamburg - Germany

Das bezeichnete Produkt entspricht den einschlägigen grundlegenden Anforderungen der aufgeführten EG-Richtlinien und Normen. Bei einer nicht mit uns abgestimmten Änderung des Produktes oder einer nicht bestimmungsgemäßen Anwendung verliert diese Erklärung ihre Gültigkeit.

The Product named below fulfills the relevant fundamental requirements of the EC directives and standards listed. In the case of unauthorized modifications to the product or an unintended use this declaration becomes invalid.

Produkttyp Product type

# Absorptionsmessgerät

Typenbezeichnung Type designation

## Absorbance 96

Einschlägige EG-Richtlinien/Normen Relevant EC directives/standards

# 2011/65/EU; 2014/30/EU;

DIN EN ISO 14971:2013-04; DIN EN 62366-1:2017-07; DIN EN 62304:2016-10; DIN EN 61010-1:2011-07; DIN EN 61010-2-101:2017-10; DIN EN 61326-1:2013-07; DIN EN 61326-2-6:2013-07

Hamburg, den 08.08.2019

Dr. Sebastian Metz

Dr. Yousef Nazirizadeh