

Instruction manual

VWR® Automated Cell Counter

EU cat. no 49862-2000



CE UK CA



Legal address of Manufacturer

Europe

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1- Safety Information

Please follow the guidelines below, and read this manual in its entirety to ensure safe operation of the unit.

1.1 General safety requirements

- a) Please follow the instructions on all safety warnings and labels in the manual and on the instrument. The instrument should only be used by professionals within the scope of application, with proper components and accessories suitable for the instrument to be used. The manufacturer will not be held liable for any damage to the instrument caused by any improper operation, including damage to any third party.
- b) Users are allowed to maintain the instrument only to the extent of recommended maintenance stated herein (Refer to Chapter -9 General maintenance). Any change to the instrument without the manufacturer's authorization may cause failure of the instrument.
- c) In case the instrument breaks down or requires any service, shut down the instrument and contact with VWR customer service.

1.2 Electrical safety

The following provisions should be followed for the safe use of the electrical devices accompanied with the instrument:

- a) Never disassemble the instrument without any authorization against any possible electric shock.
- b) Check whether the working voltage settings of all components are correct before connecting the instrument to the mains. Use the power supply compatible with the instrument and see the model label of the instrument for the compatible power supply.
- c) Connect the instrument correctly, and the cell counter need to be plugged to a conformly electric and safe system.
- d) Make sure to clean power cords.
- e) If any potential unsafe operation is detected, power off and unplug immediately to disconnect the mains.
- f) If any liquid gets contact with the circuit of the instrument, power off and unplug immediately to disconnect the mains, and wipe the moistened parts of the instrument dry in a timely manner.
- g) Keep the bench always dry during operation.
- h) The fuse type (rated voltage, rated current and model) must comply with the manufacturer's specifications. Do not use any unserviceable or used fuse, and avoid short circuit of the fuse.

1.3 Chemical waste safety

Caution! Hazardous Waste. Please refer to the SDS and relevant local regulations for handling and disposing of waste.

To minimize the risk of chemical waste, users must:

- a) Read and understand the SDS provided by the chemical manufacturer in the waste container before storage, handling or disposal of chemical waste.
- b) Provide main and auxiliary containers for the waste storage. (The main container is used to store direct waste, and the auxiliary container to store waste spilled or leaking from the main

container. Both containers must be compatible with the chemical properties of the waste materials and comply with local regulations on waste storage.)

- c) Minimize exposure to chemicals. Wear appropriate protective articles (such as protective glasses, gloves or protective clothing) for chemical handling. For detailed safety guidelines, please refer to SDS.
- d) Minimize the risk of inhalation of chemicals. Close the chemical containers after use immediately. Use chemicals only in a well-ventilated environment (e.g. fume cupboard). For detailed safety guidelines, please refer to SDS.
- e) Handle chemical waste only in a well-ventilated environment.
- f) Use the accompanying cover to seal the waste containers emptied.
- g) Disposal of waste in trays and bottles in accordance with good laboratory practices and local environmental and health regulations.

1.4 Biosafety

It is possible that biological samples (such as samples of tissues, body fluids and blood of human and animals) may transmit infectious diseases. Follow all applicable local regulations from time to time. Wear suitable protective glasses, clothing and gloves.

1.5 Certificates

Complies with the requirements of the EC EMC directive 2014/30/EU with amendments.

Test Standards:

EN IEC 61326-1: 2021 EN IEC 61326-2-6: 2021 EN IEC 61000-3-2: 2019/A1: 2021 EN 61000-3-3: 2013/A2: 2021

2- Package Contents

Configuration	Part numbers	ltems	Quantity	Purpose Description
Standard	49862-2000	VWR [®] Automated Cell Counter	1	Cell count
Standard	/	AC adapter (EU)	1	Power supply
Standard	/	AC adapter (UK)	1	Power supply
Standard 10228-0050		Cell counting slide	1	1 box of 50 pcs
Standard	/	USB flash driver	1	Data transfer

3- Intended use

This equipment is designed for measurement and analysis of cell quantity and viability for cell lines, stem cells and primary cell samples. For research only. Not intended for diagnostics use.

4- Symbols and conventions



Flammable environment hazard

Never operate the instrument in an environment with flammable gases



Electromagnetic interference hazard

Make sure to operate the instrument in a controlled electromagnetic environment to avoid any risk associated with instrument failure.



Radiation hazard

Follow all applicable radiation safety procedures all the time when handling radioactive samples. Make sure to take appropriate disinfection and safety measures during disposal of radioactive pollutants. Operators must always wear protective supplies (such as particle protective mask, gloves, protective shoe covers) according to the relevant rules and regulations of the respective laboratories for the disposal of radioactive pollutants which must be performed in accordance with the relevant regulations.

Biological infection hazard

Samples used in the intended operation of the instrument may be infectious. For this reason, it is recommended to follow the general laboratory regulations on infection control procedures. For information on decontamination agent, its use, dilution and effective application, please refer to the Laboratory Biosafety Manual issued by the World Health Organization in 2020. Follow all applicable safety procedures all the time when handling infectious samples. Make sure to take appropriate disinfection and safety measures during disposal of infectious substances. Operators must always wear protective supplies (such as particle protective mask, gloves, protective shoe covers) according to the infection control procedures of the respective laboratories, and infectious pollutants must be disposed of in accordance with the relevant regulations.

5- Product Specifications

Parameter	Description
Cell size range	Recommended 7-60 µm, allowable 4-60 µm
Cell circularity range	1-100 (min./max. size)
Cell concentration range	10 ⁴ -10 ⁷ cells/mL
Cell counting time	<9 s
Accuracy of multi-recognition	CV<5%
Built-in memory	1,000 entries
Touch screen resolution	1024*600
Operation response time	100 ms
Optics	5 megapixel camera, 2.5X optical magnification

6- Instructions for use

6.1 Overview

In the process of cell culture, it is necessary to determine the cell concentration and identify the cell viability to guide the subsequent experiments.

The automated cell counter collects and analyzes cell images and then obtain the concentration of the sample. After trypan blue staining, the proportion of living/dead cells can be obtained.

6.2 Description of Buttons and Switches



Fig. 6-1



- ① Touch screen display: 7-inch capacitive screen displaying user operation interface.
- 2 POWER Indicator.
- ③ USB port 1: allows users to download the cell count data and image onto an external device with the USB port for storage. USB flash driver format: FAT32.
- ④ Counting slide port: allows users to insert the counting slide loaded with samples.
- \bigcirc USB port 2 : the function is the same as USB port 1.

Note: two USB ports are provided at the front and rear of the instrument for data transfer, and the instrument does not support reading of two USB flash drivers simultaneously.

- (6) Quick knob on rear cover plate: users are allowed to loosen two quick knobs by counter clockwise rotation.
- \bigcirc Rear cover plate.
- 8 Power switch: main power switch.
- 9 Power input: users must use provided power cord and proper plug in compatible with the socket in user's country to connect the instrument to the socket by the power input.

7- Operation

7.1 Start-up

When the instrument is properly wired, users are allowed to start up the cell counter. Press the power switch to start up the instrument and enter the main interface as shown in the picture below.



Fig. 7-1

The main interface contains four parts: System Settings, Previous Counts, Profiles, and Count.

7.2 Profiles

In the BF assays, users are allowed to set the threshold values of the count results based on the profile sliders of size, brightness and circularity; in the FL assays, users are allowed to adjust the RFU slider as required to set the threshold values of the count results.

The Profiles interface allows users to adjust the threshold values of the profiles to be used, rename and save them for calling of such adjusted Profiles in the next cell counting.

Bright field (BF) Mode

Click [Profiles] on the main interface to enter the profiles setting interface, with the instrument in Non-Trypan Blue (Non-TP) mode by default under which the total cell concentration, histogram of total cell size distribution histogram and average size are available for view.



Fig. 7-2

Click [Trypan Blue] (TP) to switch to Non-TP under which the total cell and dead/ live cell concentrations, histograms of dead / live cell size distribution and average size are available for view.



Fig. 7-3

• Parameter adjustment



Size, brightness and circularity sliders

Size: drag the slider to enable the system to screen the cells by the size while counting.



🐸 = Smaller target

Size screening: allowable range of 7-60 µm by default, the lower limit of identification up to 4µm;

When there are much debris in the sample, the interference of cell debris can be eliminated by adjusting the recognized size range and setting the threshold value.

Brightness: drag the slider to enable the system to screen the cells by brightness while counting.



Circularity: drag the slider to enable the system to screen the cells by circularity while counting.



The circularity slider can only be used to set a single threshold value; cells within the set value range will be counted, excluding the cells beyond the range. Drag the slider toward the target to adjust the circularity threshold.

Click or hold down the above icon to adjust each parameter threshold.

Click the [Help] symbol 2 in the upper right corner of the interface to learn more about the concepts of size, brightness, circularity and so on, as shown in figure 7-4.

Larger	Smaller	High RFU	C Low RFU
Size: Take the objects within the size range into account.		RFU: Take the objects fluorescence uni account.	within the relative ts(RFU) range into
small cell debris	he size range can be adjusted to filter out mall cell debric		utomatically adjust RFU is the effect of background counting
O Less circular	O More circular	Brighter	dimmer
Circularity: Take the objects v range into accoun	vithin the circularity it.	Brightness: Take the objects range into accou	within the brightness int.
The sample may contain irregular debris that cannot be filtered by size, and circularity can be adjusted down to exclude irregular debris		Cells may be at diffe different brightness cells at different foo range is foll.	rent focus levels and show The algorithm can count us levels when the brightne

Fig. 7-4

Quick Mode

By checking [Quick Mode], you will perform count in a quick mode; otherwise, you will perform count in a standard mode. After selecting the count parameters in the Quick mode, once the counting slide is recognized, the number pad pops up for user to enter a Profile name, and then the system will count the cells and output the results; in the Standard mode, the focusing results will be presented first, and then the count results will be output after manually clicking the [Count] key. The quick mode is suitable for cell samples with no debris and moderate concentration. It can automatically focus and count after inserting the counting slide, which can greatly reduce the counting time. Even if the imaging effect in the counting results is not ideal, you can click the back key to enter the Count interface again for manual fine-tuning.

Auto Exposure

Choose by yourself whether to activate [Auto Exposure]. The Auto Exposure features allows you to adjust the brightness depending on the characteristics of cells for count, and then choose whether to restore the system to the default brightness after count.

Autosave results

When [Autosave results] is checked, the counting is complete and the counter exits, the data can be saved automatically.

Note: Autosaving succeeds only when the local storage is sufficient.

Restore to Default Mode

Click [Default] to restore part of the initial settings, including Auto FL Exposure, Auto Exposure, Quick Mode, Standard Mode, threshold value of each profiles, etc.

7.3 Profiles Loading





In the left column of the Profiles interface, you can delete/ add/ rename/ Lock profiles by clicking the

icons of the transferred to the respectively.

Note: Locked parameters cannot be edited.

Click lock key to lock the selected profile, the icon is also displayed on the right of the profile name; if you want to unlock the profile name, select it again, the lock key at the bottom becomes the

unlock key **L**, click the unlock key and confirm as prompted

Here, in addition to the Default and Red Blood Cells profiles, 16 profiles can be added at most, available for direct calling before and during count.

Each profile defines the count parameters (size, brightness, circularity) to simplify the workflow. After selecting a profile, click [Load] to apply the specified count parameter to all new cell counts. Users already completing counting, are allowed to apply the new count settings to the current count results (total cells, viability, etc.) and all new counts by loading new data from the Results interface.

7.4 Sample Preparation

The recommendations below should be followed for the best results:

- 1) Make sure to mix the cell suspension well before drawing the cell sample. Allow the cell sample to stand for a long time and then flick the centrifuge tube or gently blow the cell suspension and draw the sample from the centre of the tube rather than its bottom.
- 2) Cell concentration range: 1×10^4 – 1×10^7 cells/mL, optimally 1×10^5 – 4×10^6 cells/mL.
- 3) Make sure that the count area is covered with cell suspension to get accurate cell viability analysis results and start counting immediately after staining according to the analytical protocol.
- 4) Do not press the optical surface of the counting slide, but hold it by the edges.
- 5) Carefully avoid any bubble formed in the sample.
- 6) Minimize the amount of debris in the cell sample to the greatest extent. No matter which counting or staining method is selected, it is difficult to count and assess the viability of samples containing large amounts of debris which may come from trypan blue stain or cell samples. Possible treatments for different sources of debris are provided as follows:
 - The trypan blue stain is centrifuged to obtain the supernatant;
 - The cells are centrifuged and then re-suspended.

7.5 Methods of Staining

Tips: If you only need to count the total cell concentration, you could directly load sample without staining.

• Trypan blue staining

Recommended staining method:

- 1) Mix 10μ L cell suspension with 10μ L 0.4% Trypan blue solution.
- 2) After pipette and mixing well, transfer 10µL of stained cells into the cell counting slide for analysis.

7.6 Loading Samples onto Slide

- 1) After pipette and mixing well, transfer 10µL of sample into the cell counting slide for analysis.
- 2) Allow the sample to stand for 30 seconds on the slide, and insert the sample-loaded slide into the slide port. When correctly inserted, a soft click will sound.
- 3) Just push the counting slide gently into the slide port until it clicks and is pushed out under spring pressure. Hold the counting slide by the edges to take it out.

Note: do not reuse the disposable counting slide, which may influence the count results.

7.7 Bright Field Assays

If the system recognizes insertion of the counting slide, the file name editing interface will pop up. Shown as figure 7-6.



Fig. 7-6

Enter the file name and click [Enter] or click [skip] ("skip" means that the default name or last typed name is selected) to enter the count interface. Here you could start counting and perform such actions as zooming in, fine tuning of focus and light source brightness adjustment, so as to capture optimized images for accurate count of cell concentration or dead/ live cells.

Count page of TP / Non-TP



Fig. 7-7

111 Default

in the figure above shows the active profile file name. Click this icon to enter the Profiles page.

1x For setting the dilution factor of the sample. Click"1×"to pop up a numeric keypad. The range of setting value: 1.00~999.00.

Note: The default dilution of Trypan Blue mode is "2×".

Dilution factor of sample:

The cell concentration counted by cell counter is the measured concentration of the cells on the current counting slide, which may be accessible from the dilution of the original solution; so users need to convert the current concentration into the concentration of the original solution to calculate the volume of the original solution required for dilution to the target concentration.

allows users to adjust the light source brightness. If users check [Auto Exposure], the results will be auto adjustment values by default. Users can also manually adjust the brightness of light source by

clicking 🔲 & 🛨. Adjustment range: 0-100.

Brighter or dimmer effect may have an effect on the imaging quality. In general, when automatic exposure is activated, brighter field white light illumination can be obtained automatically on the imaging interface. The overexposed, moderate and underexposed effects of the bright field are as shown in Fig. 7-8 (A-C) below



В



A Overexposure: brighter light source, whiter background, and the cell details shaded by light, affecting cell recognition.

B Underexposure: dimmer light source, darker background, and darker cells as a whole, affecting the judgment of the live and the dead cells.

C Normal exposure: moderate brightness of light source, clear outline of cells, live and dead cells showing significantly different transmittance characteristics.

Focusing:

Click 😐 to

When the counting slide is inserted, the system recognizes the counter and starts focusing automatically.

	1230			· · · · · ·	
	•	-	6	+ Set Aut	
pop up the slide bar:	¥.				

Click [Auto] to execute auto focus.

Click and to adjust the focus. Focus value is displayed in real time, such as "1230" showed above the bar. Adjustment range of aggregation value: 0-2400.

The mean of [Set]: customize the focus value. The next time the instrument is used, the system will focus based on the focus value of the last "Set" in order to quickly obtain good pictures for accurate counting.

Note: the point of set will be influential to autofocus.

Focusing recommendations:

It is critical to ensure proper focus to accurately identify cells and distinguish dead or live cells. Cell images with correct and incorrect focus are as shown in Figure 7-9(A-B)



A Normal focus: well-outlined cells, with a clear aperture in the middle of live cells, and opaque dead cells due to trypan blue staining.

B. Abnormal focus: blurred cell contour, presented as opaque dead cells.

Two possible results from incorrect focusing:

- Due to the spherical structure of the cell, the cell center will present as opaque after as if it had been stained with Trypan blue in the event of deviation of the focal length of the device, leading to the misjudgment of live cells as dead cells by the device.
- The focal length of the device is deviated, and the cells presented a fuzzy halo, which causes the device to misjudge the dead cells as live cells.

Determination of correct focusing:

- After automatic focusing, observe the imaging state of the cells in the field of vision. If some cells show clear outline and is transparent in the middle part, and some cells are clear as a whole but opaque in the middle part, then the cell focus is correct.
- If there are no obvious features mentioned above, appropriately enlarge the image and select the area with a large quantity of cells, and slightly adjust the focal length to observe the changes of cell imaging in a clear manner when the focal length changes, and select the state with the clearest cell edge as the focus.



to zoom in/out the active view/ image.

Upon completion of operations on the [Count] page, the [Result] page will pop up, where users are allowed to zoom in, mark cells, or view histograms, adjust profiles and count for the captured images. The results page of TP Mode as shown below.





Click to pop up a histogram, as shown in the figure below, and click it again to exit.





Click to mark cells, as shown in the Fig.7-12 below.



Fig. 7-12

Click 📟 to enter the Adjustment page to re-adjust the profiles.

Click to enter the Dilution Calculator page, as shown in the figure below, where users are allowed to calculate the volume of existing cell stock solution and the volume of buffer solution to be mixed and diluted to the target volume and concentration.

is a "Help" symbol on the upper left corner of the interface; click it for more about calculation method.



Fig. 7-13

7.8 Results Storage

After counting, users are allowed to save or export the original image, cell marking image, count profiles, cell count report and other data, and are also allowed to preset the default naming rules for results exporting.

Click [Save as] to save the results to the local computer in various forms or/ and to an acceptable external storage device. Click [Done] to enter the [System Homepage] and automatically save the results to the local computer or/ and external storage device under the system default name.

Note: 1000 entries are available for storage in the system at most. If users want to save new data beyond this limit, the earliest count results saved will be deleted by default.

5 Sa	ve as	11:36 2023-02-07
File Name Red blood Save to local storage (including)	PDF report & tagged image)	
Save to external storage device	✓Original pic JPg ✓ ✓Tagged pic Png ✓	☑ Data .CSV ☑ Report.PDF
		ОК

Fig. 7-14

7.9 Previous Counts

Click [Previous Counts] on the Homepage to enter the following interface, where users are allowed to view the previous data of the instrument operation, delete and export data to the external storage device.

5		Previous Count	s	11:36 2023-02-07
	🍞 🗕 Expiration time	R		Q
All (select 0 it	File Name	Date & Time	Total concentration	
1	Default	2023-02-03 11:18:15	2.12 x 10 ⁵ /mL	
2	Default	2023-02-03 11:18:03	2.07 x 10 ⁵ /mL	^
				001/001
				\sim
ŝ	Delete	G Export	Kiew	
		C : 7 4 C		

Fig. 7-15

7.10 System Settings

Click [System Settings] on the Homepage to enter the following interface. It allows users to perform such actions as software update, date / time setting, language switching.

Note: "Maintenance" is only for after-sales personnel.



Fig. 7-16

• Software update

The latest version of the software is available for updating to the instrument by the USB flash drive. Method: First copy the latest software to the USB flash drive, insert the USB flash drive into the USB port, when the USB flash drive is successfully recognized, you could click [Update Now]. When the software program in the USB flash drive is successfully recognized, the keyboard pops up. After the user enters the correct password, the software update interface is displayed. Please follow the

prompts. Note: the .CSV data in the USB flash drive will be automatically deleted after upgrading. It's recommended to back up the data to the PC before the upgrade.

Ś	Software Update	11:31 2023-02-07
	App Version: V01.00.00.9444 BSP Version: V01.00.00.9010	
	Update Now?	
	Cancel Updat	e Now

Fig. 7-17

8- Troubleshooting

Review the information in the table below to troubleshoot operating problems. For application question, please contact <u>TechnicalProductSupportEU@vwr.com</u> (English only).

Fault/alarm	Solution			
Autofocus fails to focus cells well	Make sure there is no bubble or debris on the screen to interfere with autofocus.			
The USB flash drive failed to be recognized	 The USB flash driver is not in FAT32 format. Format it into FAT32. Replace a USB flash driver in the correct format. 			
Firmware update fails	 Make sure the USB flash driver is in FAT32 format, and upload the file to the USB flash driver for software update. The file must be saved into the root directory of the USB flash driver instead of saving it in a folder or subfolder. The file must be intact with the extension .zip. during transmission. 			
Alarm prompt "Motor jammed, please contact the after-sales personnel!" pops up	Power off the device and contact local Avantor services or visit <u>www.vwr.com</u>			
Alarm prompt "Camera error, please contact after-sales personnel!"	Power off the device and contact local Avanto services or visit <u>www.vwr.com</u>			

9- General maintenance

1) Touch screen cleaning

- Gently and carefully clean the touch screen with a soft lint free cloth moistened in an LCD cleansing detergent. Wipe the touch screen dry immediately after cleaning.
- Prevent ingress of cleaning solvent into the power button, power socket, counting slide port or USB port.
- Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.
- Never use any corrosive cleaning solution against any scratch on the touch screen.

2) Case cleaning

- Clean the case with a soft lint free cloth moistened in distilled water=. Wipe it dry immediately after cleaning.
- Prevent ingress of water or other cleaning solvent into the Power button, power socket, counting slide port or USB port.
- Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.

3) Decontaminating

- Clean the outer case with a soft lint free cloth moistened in 70% alcohol. Wipe it dry immediately after cleaning.
- Never use a bleach solution that may cause bleaching liquid crystal on the instrument.
- Prevent ingress of water or other cleaning solvent into the power button, power socket, counting slide port or USB port.

Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.

10- User replaceable accessories and spare parts

Description	Quantity	Cat. No.
AC adapter (EU)	1	/
AC adapter (UK)	1	/
Cell counting slide	1 box of 50 pcs	10228-0050

11- Technical service

Visit the VWR website at www.vwr.com for:

- Complete technical service contact information
- Access to the VWR Online Catalogue, and information about accessories and related products
- Additional product information and special offers

Contact us For information or technical assistance contact your local VWR representative or visit <u>www.vwr.com</u>.

12- Warranty

VWR warrants that this product will be free from defects in material and workmanship for a period of two (2) years from date of delivery. If a defect is present, VWR will, at its option and cost, repair, replace, or refund the purchase price of this product to the customer, provided it is returned during the warranty period. This warranty does not apply if the product has been damaged by accident, abuse, misuse, or misapplication, or from ordinary wear and tear. If the required maintenance and inspection services are not performed according to the manuals and any local regulations, such warranty turns invalid, except to the extent, the defect of the product is not due to such non-performance.

Items being returned must be insured by the customer against possible damage or loss. This warranty shall be limited to the aforementioned remedies. IT IS EXPRESSLY AGREED THAT THIS WARRANTY WILL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND IN LIEU OF THE WARRANTY OF MERCHANTABILITY.

13- Compliance with local laws and regulations

The customer is responsible for applying for and obtaining the necessary regulatory approvals or other authorisations necessary to run or use the Product in its local environment. VWR will not be held liable for any related omission or for not obtaining the required approval or authorisation, unless any refusal is due to a defect of the product.

Equipment disposal



This equipment is marked with the crossed out wheeled bin symbol to indicate that this equipment must not be disposed of with unsorted waste.

Instead it's your responsibility to correctly dispose of your equipment at lifecycle -end by handling it over to an authorized facility for separate collection and recycling. It's also your responsibility to decontaminate the equipment in case of biological, chemical and/or radiological contamination, so as to protect from health hazards the persons involved in the disposal and recycling of the equipment. For more information about where you can drop off your waste of equipment, please contact your local dealer from whom you originally purchased this equipment.

By doing so, you will help to conserve natural and environmental resources and you will ensure that your equipment is recycled in a manner that protects human health.

Thank you

Appendix — Our local offices in Europe, AMEA and North America

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