

# EZ–Fluo<sup>™</sup> Rapid Detection System

User Guide



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# Introduction

EZ-Fluo<sup>™</sup> System is a rapid fluorescence-based technology for fast quantitative detection of contaminants over a broad range of filterable matrices.

This easy-to-use and simple system can detect viable microorganisms filtered on a membrane down to one CFU per sample. The non-destructive method allows the identification, by any method, of any microorganisms detected during the initial fluorescence reading. Sample preparation is performed using Microfil<sup>®</sup>, EZ-Pak<sup>®</sup>, and S-Pak<sup>®</sup> filtration systems and membranes that ensure consistent and reliable results.

#### **Chemistry of the Reaction**

The EZ-Fluo<sup>™</sup> system uses proven technology based on a universal enzymatic fluorescent staining of viable microorganisms.

After a filtration step, all microorganisms retained on the membrane are stained by a fluorescence viability marker. The reaction consists of an enzymatic cleavage by active microbial metabolism of a nonfluorescent substrate. Once cleaved by the cell's metabolism, the substrate liberates free fluorochrome into the microorganism cytoplasm that remains in the cell due to its hydrophilic nature.

The signal is thereby naturally amplified by accumulation of the fluorochrome inside the cells and can be easily detected by exposure to the excitation wavelength of the fluorescence dye in the EZ-Fluo<sup>™</sup> Reader and visually counted or counted using the EZ-Fluo<sup>™</sup> Camera and dedicated software.

#### **Principles of the Procedure**

The procedure is easy and requires limited training and skills: sample contaminants are collected by membrane filtration using the Microfil® Filtration System.

After a short incubation time, media plates are removed from the incubator. Each membrane is then transferred onto a cellulose pad that has been pre-soaked with staining solution. These are then put in the incubator for 30 minutes.

Fluorescent micro-colonies can be counted two ways: directly through the viewing window of the EZ-Fluo<sup>™</sup> reader, or using the EZ-Fluo<sup>™</sup> Camera and dedicated software, which projects the image onto a computer screen. After reading, membranes can be re-incubated on media for later collection and identification of microorganisms if contamination of the sample was detected.

# **Operator and Equipment Safety**

- Read and understand this manual before using the EZ-Fluo<sup>™</sup> Reader and accessories. Failure to follow operating instructions could result in user injury or damage to the instruments.
- Read and understand all maintenance instructions in this manual before performing maintenance on the EZ-Fluo<sup>™</sup> Reader and accessories.
  Failure to follow instructions could result in user injury or damage to the instruments.
- Any alteration of the EZ-Fluo<sup>™</sup> Reader or accessories from factory specification may cause unsafe conditions, and will void the product warranty.
- Any attempt to use the EZ-Fluo<sup>™</sup> Reader or accessories in a manner not specified in this user guide may result in operator injury or damage to the instrument, and will void the product warranty.
- Use appropriate personal protective equipment, including eye protection, when operating the system.
- Do not attempt to open and repair the EZ-Fluo™ Reader or Camera. Service should be performed by trained and authorized personnel only.
- Preferably use the following components: Microfil<sup>®</sup>, EZ-Pak<sup>®</sup> and S-Pak<sup>®</sup> filtration systems and membranes; Petri-Pad<sup>™</sup> Petri Dish; Filter forceps; EZ-Pak<sup>®</sup> Membrane Dispenser; Microfil<sup>®</sup> funnel dispenser; EZ-Stream<sup>™</sup> Pump.

- Do not expose the EZ-Fluo<sup>™</sup> Reader and Camera to liquid. If this happens, turn the unit off immediately and disconnect the power supply.
- Do not spray cleaning agents directly inside the EZ-Fluo<sup>™</sup> Reader.
- Before performing any servicing activity, disconnect the EZ-Fluo<sup>™</sup> Reader power cord from the power supply outlet of the EZ-Fluo<sup>™</sup> Reader.
- The power supply must be protected by a fuse below the main connection.
- The electrical installation must comply with local standards.
- Use an electrical surge protector to prevent damage to the system.
- Locate the EZ-Fluo<sup>™</sup> Reader on a clean, flat, stable, horizontal surface, away from any source of excessive heat and close to an easily accessible, properly grounded power supply outlet.
- Do not locate the EZ-Fluo<sup>™</sup> Reader under direct lab light, as this could interfere with the reading of the membrane.

# Specifications and Operating Requirements

Dimensions	Component		Length		Width			Height	
(nominal)	Reader		24.9 cm (9.8 in.)		14.2 cm (5.6 in.)		) .	12.5 cm (4.9 in.)	
	Camera		9.7 cm (3.8 in.)		6.6 cm (2.6 in.)			11.4 cm (4.5 in.)	
Weight	Component		Weight						
(nominal)	Reader					4.	4 kg (9.7 l	bs.)	
	Camera				0.5 kg (1.1 lb.)			b.)	
Voltage	Component	Component			Supply Voltage Input Supply Voltage Ou			y Voltage Output	
	Reader			10 50	0V – 240 V Hz – 60 Hz		24V	24V direct current	
	Camera	Camera			by the comp	uter			
Power	Component		Сог	nsumption			P	ower	
	component	N	Vominal	N	laximal	No	minal	Maximal	
	Reader	6	670 mA	20	080 mA	16	watts	50 watts	
	Camera	2	200 mA	4	30 mA	1	watt	2.15 watts	
	NOTE: The reader	r is supp	plied with	its power	cord accordi	ng to re	gion.		
Environmental Operating Temperature	15 °C to 40 °C (59	15 °C to 40 °C (59 °F to 104 °F)							
Humidity	< 90 %							-	
Altitude	< 3,000 m (9,842 ft.)								
Regulatory Information	We certify that the EZ-Fluo <sup>™</sup> Reader and the EZ-Fluo <sup>™</sup> Camera were designed and manufactured in application of the following European Council directives:								
	Electromagnetic compatibility 2004/108/EC  Restriction of the use of certain Hazardous Substances in electrical equipment (RoHS) 2002/95/EC								
Materials of	Component	Component Materials of Construction							
Construction	Reader								
	Housing Painted aluminum sheet 1050								
	Optical chamber	er 304L stainless steel							
	Handle	30	04L stainl	ess steel					
	Optical filter	GI	ass						
	LED protector	Tra	ansparen	t polycarbo	nate				
	Foot	Po	olyvinylch	loride (PVC	)				
	Labels	Po	olyester						
	Screen Protector polyester								
	Camera								
	Front plate	30	04L stainl	ess steel					
	Housing	Housing Painted aluminium alloy							
	Window Glass								
	Light Guard Clear polymethyl methacrylate (PMMA)								

# System Components



### **Recommended equipment**



### The EZ-Fluo<sup>™</sup> Reagent Kit

The EZ-Fluo<sup>™</sup> Reagent Kit consists of reconstitution reagent, fluorescence reagent, staining buffer, plastic storage bag, and transfer pipettes.

The kit components must be stored at 2 to 8 °C. One EZ-Fluo™ Reagent Kit contains sufficient material for 57 tests.



# Installing the EZ-Fluo<sup>™</sup> Reader

The EZ-Fluo<sup>™</sup> Reader weighs 4.4 kg (9.7 lbs) and is designed for use on a laboratory workbench.

- 1. Remove all components and their accessories from the box.
- 2. Remove the reader from the protective bag and place it on a flat, stable, horizontal surface.

#### NOTE

Please retain all packing materials. If the unit needs to be shipped, it should be packed in the original materials.

3. Remove the protective film from the window.



4. Connect the end of the external power supply cord to the power outlet on the reader.



5. Connect the power cord to the external power supply. Connect the power cord to a power source.



The system starts up. The auto test sequence runs and verifies that the EZ-Fluo<sup>™</sup> Reader is ready to use:



# Installing the Camera (Optional)

### Installing the Camera onto the Reader

Place the camera on the top of the EZ-Fluo<sup>™</sup> Reader as shown here. The camera magnetically locks onto the reader.



### Installing the Software onto the Computer

System and Software Requirements

- 2 USB ports
- Minimum 512 MB of RAM memory; 1 GB recommended
- Minimum disk space: 500 MB
- Processor recommended: Intel<sup>®</sup> Core<sup>™</sup>2 Duo
- Minimum screen resolution 1024 x 768 recommended: 1280 x 1024
- Operating system: Microsoft Windows XP® 32 bits and 64 bits Microsoft Windows Vista® 32 bits and 64 bits Microsoft Windows 7® 32 bits and 64 bits
- Microsoft .NET Framework 2.0 SP2 is also recommended (Note: this is installed automatically during the installation of the EZ-Fluo<sup>™</sup> Counter Software)
- Adobe Acrobat<sup>®</sup> Reader 9.0 or higher to display the user guide

#### NOTE

Administrator access to the computer is required to install the EZ-Fluo<sup>™</sup> Spot Counter software. All screenshots shown are examples and may vary from one computer to another.

- 1. Switch on the computer.
- 2. Log on with an administrator account.
- 3. Insert the installation disc into the CD drive of the PC.
- 4. Using the file explorer, browse to the CD-Rom and double-click the Setup.exe file to start the installation.
- 5. The installation wizard for EZ-Fluo<sup>™</sup> Spot Counter displays. Click Next.



6. Select the language for the software. Select the destination folder for the software. Click Next.

Select software language :		•
Software selected language : Eng	ásh	
Select your destination folder (		
E Documents and	Settings	-
1306		1.1
H Stermec		_
E D NceOrivers		
E C Program Files		

7. A new window displays, listing the components required to complete the EZ-Fluo<sup>™</sup> software installation. Click **Install.** 

z Fluo Spot Counter Setup	
Requirements instalation	
E2 Fluo Spot Counter requires that computer prior to installing this appli	the following requirements be installed on your cation
Windows Installer 3.1 already i Framework Dothet 2.0 SP2 is n	installed equired
Camera driver is required Camera ActiveX is required	
Click Install to begin installing these	requirements
	Install

8. When the installation of required components is completed, click Next.

🐻 Setup - EZ-Flu	uSpotCounter			
Ez-Fluo Spot Requirement	Counter Setup Its installation			M
EZ Fluo Spo computer p	t Counter requires that the follor for to installing this application	lowing requirement:	i be installed on you	£
Windo Framer Camer Camer	vs Installer 3.1 already installe vork DotNet 2.0 SP2 installed a driver installed a ActiveX installed	đ		
Operation o	ompleted. Click Next to continu	ø.		
			146F	1
			Next >	Cancel

9. A message indicating that the software is now ready to be installed displays. Click Install.



10. A message indicating that the computer must be restarted in order to complete the installation of the software displays. Save and close all the other applications. Then, select **Yes**, restart the computer now and click **Finish**.



11. After the computer has restarted, the EZFluoSpotCounter icon is displayed on the desktop.



### NOTE

Do not open the EZ-Fluo<sup>™</sup> Spot Counter software before installing the EZ-Fluo<sup>™</sup> Camera.

1. Connect the small end of the USB cable to the camera.



2. The USB cable is equipped with two plugs. Connect the main plug to a USB port of the PC. Connect the second plug to another USB port of the PC, if available.



#### NOTE

The main plug of the camera USB cable must be connected to the same USB port during installation and use or the PC will not recognize the camera.

- 3. The PC detects the EZ-Fluo<sup>™</sup> Camera and automatically installs some components. Wait until installation is finished.
- 4. The installation is complete.

# Preparing the Reagent Kit

### **Precautions and Methods**

- Do not use any component in the kit that has expired.
- Remove the kit from the refrigerator one hour before starting the reconstitution.
- Reconstitute in a controlled-area environment.
- During the seven-day shelf life, do not let the Staining Solution remain at room temperature for more than four hours per day.
- After removing the Staining Solution from the refrigerator, wait one hour before performing a staining.
- Use appropriate personal protective equipment when using the Staining Solution.
- Mix the Staining Solution before performing the first test of the day.

#### **Reconstituting the Staining Solution**

Reconstitute the Staining Solution following the procedure on the Staining Buffer vial (vial 3) before the first use of the reagent kit. The reconstitution date may be noted on the Staining Buffer (vial 3) label.



Store the reconstituted Staining Solution at 2 to 8 °C. One EZ-Fluo<sup>™</sup> Reagent Kit contains sufficient material for 57 tests. The approximate number of remaining tests is indicated on the Staining Buffer (vial 3) label. The shelf life of the reconstituted Staining Solution is seven days.

#### NOTE

To extend the shelf life, the reconstituted Staining Solution can be distributed in up to four aliquots. These aliquots can be stored up to one month in the freezer. Once thawed, the aliquot must be stored at 2 to 8 °C, and protected from light. The shelf life of the thawed aliquot is seven days.

# Using the Traceability Labels (optional)

1. Record the test information on the traceability labels. Use the Label Printing Software to print data on the traceability labels. Please refer to the Label Printing Software User Guide.

### NOTE

To download this software and user guide, go to www.millipore.com and search "Label Printing Software".

2. Before the sample filtration, apply the label to the bottom of the plate containing the media that will be used for the test.



3. Before the staining, remove one part of the label by grasping the blue arrow side with forceps. Place the label at the bottom of the Petri-Pad<sup>™</sup> Petri dish that will be used for the staining step.



4. After the reading step, if reincubation is necessary, transfer the traceability label from the bottom of the plate used for the incubation to the bottom of the plate that will be used for reincubation.



# Filtering the Sample

- Perform the sample filtration using Microfil<sup>®</sup> Filtration System as described in the Microfil<sup>®</sup> & S-Pak<sup>®</sup> Membrane Filters/Microfil<sup>®</sup> & EZ-Pak<sup>®</sup> Systems User Guide. The optimal vacuum is obtained using the EZ-Stream<sup>™</sup> Pump (see EZ-Stream<sup>™</sup> Pump User Guide).
- 2. Transfer the membrane into a plate that contains medium. Ensure that the membrane is distributed directly and uniformly on the medium to avoid the formation of wrinkles and air bubbles.



3. Place the lid on the plate.

# Incubating the Membrane

Place the plate that contains the membrane into the incubator, with the plate lid facing down.



# Staining the Membrane

- 1. Remove the plate that contains the membrane from the incubator and transport it into a controlled environment area.
- 2. Remove the lid of a Petri-Pad<sup>™</sup>.
- 3. Using a pipette, add 1.7mL of Staining Solution on the center of the pad of the Petri-Pad<sup>™</sup>.



#### NOTE

If necessary, continue adding small amounts of Staining Solution until the pad is saturated.

4. Place the lid on the Petri-Pad<sup>m</sup>.



- 5. Remove the lid of the plate that contains the membrane.
- 6. Using forceps, separate the membrane from medium.



7. Open the lid of the Petri-Pad<sup>™</sup> that is wetted with Staining Solution.

8. Transfer the membrane into the Petri-Pad<sup>™</sup>. Ensure that the membrane is distributed directly and uniformly on the medium to avoid the formation of wrinkles and air bubbles.



- 9. Close the lid.
- 10. Incubate the Petri-Pad<sup>™</sup> that contains the membrane for 30 minutes at 32.5 ± 2.5 °C with the Petri-Pad<sup>™</sup> lid facing down.





To ensure optimal results, do not stack multiple Petri-Pad<sup>™</sup> Dishes in the incubator.

# Installing the Membrane in the Reader

- 1. After the staining step, remove the Petri-Pad<sup>™</sup> that contains the membrane from the incubator.
- 2. Verify that the reader is on and ready to use.
- 3. Open the reader drawer and place the Petri-Pad $^{\mathrm{m}}$  in the drawer.





4. Close the drawer.

# **Reading the Membrane**

- 1. When the reader drawer is closed, the reader lights the membrane.
- 2. Read the membrane two ways:
  - Read the membrane manually, directly through the reader viewing window
  - Read the membrane using the EZ-Fluo<sup>™</sup> Camera and EZ-Fluo<sup>™</sup> Spot Counter Software
  - This section contains instructions for both methods.

#### NOTE

If the EZ-Fluo<sup>™</sup> Reader drawer remains closed for 10 minutes, and there is a Petri-Pad<sup>™</sup> inside, the LED lights inside the reader switch off and the system enters standby mode.



To relight the membrane, open and close the drawer. This resets the counter on the reader screen.

If the EZ-Fluo<sup>™</sup> Reader is not used for more than 30 minutes, and there is no Petri-Pad<sup>™</sup> inside, the reader goes into sleep mode. To reactivate the reader, push the counting button or open the drawer and insert a Petri-Pad<sup>™</sup> containing a membrane. Close the drawer. When the reader is reactivated, the auto test sequence runs.

#### Reading the Membrane manually

To read the membrane manually, view the membrane directly through the reader viewing window.

1. Push the counting button to increase the count. The screen on the reader displays the counter.



#### NOTE

To make counting easier, align the membrane grid with the drawer.

2. After the counting is complete, record the count and open the drawer to remove the Petri-Pad<sup>™</sup> from the reader.

#### NOTE

Five seconds after the drawer opens, the counter disappears and the message Waiting for Petri-Pad<sup>™</sup> is displayed on the screen.



3. Close the drawer.

#### NOTE

In case of use of black membrane, do not use the camera to read the membrane

#### Starting the EZ–Fluo<sup>™</sup> Spot Counter Software

1. Double-click the 💭 icon on the desktop. The software opens.



#### NOTE

If the EZ-Fluo<sup>™</sup> Camera is not connected to the PC, the message below displays before the opening of the software.

uEye	Cam	×
No	uEye camera pre	esent
	ок	

In order for the computer to recognize the camera, the camera must be connected to the same USB port during use that it was connected to when it was installed.

### Capturing an Image

- 1. Place a Petri-Pad<sup>™</sup> containing a membrane into the reader.
- 2. In the menu, click Capture (or press Ctrl + space).

### NOTE

During the image capture, a progression bar displays and the camera LED light is orange. A blinking orange LED light indicates an error. 3. The membrane image is displayed.



#### **Counting the Spots**

- 1. Mouse over the membrane image. The cursor changes to a cross.
- 2. Using the mouse, place the cross on a spot.
- 3. Left-click to mark the spot and increase the counter reading. The counter is located on the screen beneath the words "EZ-Fluo<sup>™</sup> Spot Counter."

#### NOTE

To delete a marking, place the cursor on the spot you want to delete and right-click.

### Changing the Size of the Image

To increase the size of the image, use the scroll wheel of the mouse.

### Changing to Full Screen Mode

1. In the menu, click **FullScreen**. The counter value and the zoom factor display in the menu. Click **FullScreen** again to return to normal display mode.



### Changing the Pen Size

1. In the menu, click Pen size.

Pen size	Ful	lScreen	Extrapola
Small		Ctrl+Nu	mPad1
Mediu	m	Ctrl+Nu	mPad2
Large		Ctrl+Nu	mPad3
Cross		Ctrl+Nu	mPad4

2. Select the pen size you want to use. Several pen sizes can be used to mark the spots of one membrane.

#### NOTE

The standard pen size is Medium.

#### Counting by Extrapolation

When there are too many spots on the membrane to count one by one, use the Extrapolation function to estimate the number of spots contained on the entire membrane.

#### NOTE

When using the extrapolation function, mark spots only within the outlined segment. Activating the extrapolation function resets the counter.

- 1. In the menu, click **Extrapolation.** A one-eighth segment of the membrane is outlined.
- 2. Using the method outlined above, count the spots in the outlined segment.



The extrapolated number of spots for the entire membrane is indicated at the top of the screen, in the menu.

File Capture Reset Pen size FullScreen Extrapolation = 208 Help Count : 26 Zoom : 100%

3. Click Extrapolation again to quit this function.

In the menu, click Reset to erase all markings and reset the counter.

#### Saving an Image

1. In the menu, click File. Select Save as.

File	Capture	Reset	Pen size	Fu
C	pen		Ctrl+O	
S	ave as		Ctrl+S	
P	rint	- 0	Ctrl+P	
P	rint with ove	erlay	Ctrl+Shift+P	
E	xit		Ctrl+Q	

- 2. Select the folder you want to save the image in.
- 3. Select the format for the saved image (.bmp or .jpg).

### NOTE For best image quality, choose .bmp format. Images are saved without the counting marks.

- 4. Enter the picture name in the File name field.
- 5. Click Save.

#### Printing an Image

Images can be printed with or without counting marks.

- 1. In the menu, click File.
- 2. To print the image without counting marks, select Print.

File	Capture	Reset	Pen size	Fu
C	)pen		Ctrl+O	
s	ave as		Ctrl+S	
P	rint	R.	Ctrl+P	
P	rint with ov	erlay C	trl+Shift+P	
E	xit		Ctrl+Q	

3. To print the image with counting marks, select **Print with overlay.** 

File	Capture	Reset	Pen size	Fu
C	pen		Ctrl+O	
s	ave as		Ctrl+S	
P	rint		Ctrl+P	
P	rint with ov	verlay <mark>∖</mark> C	trl+Shift+P	
E	xit	.,	Ctrl+Q	

#### Opening a Saved Image

#### NOTE

Once the image opens, the image functions are available. The EZ-Fluo™ Camera does not need to be connected to open and work on a saved image.

1. In the menu, click File. Select Open.

File	Capture	Reset	Pen size	Fu
C	pen	he la	Ctrl+O	
S	ave as		Ctrl+S	
P	rint		Ctrl+P	
Р	rint with ov	erlay C	trl+Shift+P	
E	xit		Ctrl+Q	

2. Navigate to the file to be opened.

### Using Help Menu

In the menu, click Help and select Documentation to access the .pdf version of this user guide.

Help About Documentation

### Closing the EZ-Fluo<sup>™</sup> Spot Counter Software

In the menu, click File and select Exit.

File	Capture	Reset	Pen size	Fu
C	)pen		Ctrl+O	
s	ave as		Ctrl+S	
P	rint		Ctrl+P	
P	rint with ov	erlay C	trl+Shift+P	
E	xit	R	Ctrl+Q	

#### Performing a Reincubation (optional)

Follow this procedure if reincubation is necessary after the membrane is read.

- 1. Have ready a new plate that contains adequate medium.
- 2. Remove the lid from the Petri-Pad<sup>™</sup>.
- 3. Using forceps, gently lift the membrane from the pad.



NOTE Be careful to not damage the membrane.

4. Transfer the membrane into a new plate.



- 5. Place the plate that contains the membrane in the incubator, with the lid facing down.
- 6. Remove the plate with the membrane from the incubator.
- 7. Count the colony forming unit (CFUs).



8. Take sample of the colonies and identify them following the appropriate standard protocol.

# Storing the Reader

If the reader will not be used for an extended period of time, make sure the drawer is empty, close the drawer, and unplug the power cord from the power source.

# Maintenance

### Cleaning the EZ-Fluo<sup>™</sup> Reader and Camera

- 1. Disconnect the reader from the power supply before cleaning.
- 2. Use only nonwoven wipes soaked in one of the products listed below. Do not spray any cleaning products, solvents, or abrasives on or into the EZ-Fluo<sup>™</sup> Reader or Camera, as these could damage the instruments.

Cleaning Product	Dilution
Quaternary ammonium (NH4)	
Biguanide	Ready to use
Propanolol (10 to 40%)	
Isopropyl alcohol 70% (aerosol)	Ready to use
Ethanol 70%	Ready to use
Sodium Hypochlorite	250 ppm
Peracetic acid at 5%	
Acetic acid at 7%	2.5%
Hydrogen peroxide at 27%	
Ethanol 41%	
Polyhexamethylene biguanide hydrochloride	Ready to use
N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine	Ready to use
Didecyldimethylammonium chloride	

- 1. Gently clean the reader viewing window and the screen using a nonwoven wipe soaked in one of the products listed in the table in this section.
- 2. Gently clean the membrane site on the reader drawer using a nonwoven wipe soaked in one of the products listed in the table in this section.



3. Clean the window of the EZ-Fluo<sup>™</sup> Camera using a nonwoven wipe soaked in one of the products listed in the table in this section.

### Weekly Cleaning: Reader and Camera

Disinfect all the exterior surfaces of the reader and the camera using a nonwoven wipe soaked in one of the products listed in the table in this section.

### Monthly Cleaning: Reader

1. Remove the drawer from the reader by pulling firmly on the drawer.



2. Gently clean the drawer using a nonwoven wipe soaked in one of the products listed in the table in this section.

### NOTE

The drawer is not autoclavable.

3. Invert the reader. Gently clean the interior of the reader using a nonwoven wipe soaked in one of the products listed in the table in this section.



4. Replace the drawer inside the EZ-Fluo<sup>™</sup> Reader.

#### **Annual Servicing**

A complete check of the EZ-Fluo<sup>™</sup> System should be performed by one of our authorized technicians annually.

# Troubleshooting

Error Message or Symptom	Possible Cause	Remedy		
EZ-Fluo™ Reader				
The light (LED) and the screen on the	The reader is not connected to power source.	Connect to power source.		
reader are off.	The reader is connected to power source.	Contact Technical Support.		
The light on the reader is on but the screen is off.	The reader is in sleep mode.	To reactivate the reader, push the counting button or open the drawer and place a Petri-Pad™ containing a membrane inside.		
	The reader is not connected to power source.	Connect to power source.		
The membrane is not lit when the	The drawer is not properly closed.	Close properly the drawer.		
reader drawer is closed.	The Petri-Pad <sup>™</sup> moved away from the drawer membrane site when the drawer was closed.	Open the drawer and verify that the Petri-Pad <sup>™</sup> is properly located in the drawer membrane site. Close the drawer.		
The Petri-Pad™ is lit but the Millipore logo is visible instead of the membrane.	The Petri-Pad <sup>™</sup> is inserted in the reader upside down.	Invert the Petri-Pad™.		
The counting function does not work.	The counting button does not work properly.	Contact Technical Support.		
The VERIFICATION NEEDED message displays at the top of each screen.	Some of the LEDs inside the reader are not functioning properly.	Contact Technical Support.		
The CALIBRATION NEEDED	30 days before the calibration due date, the message is displayed at the top of each screen.	Contact us to calibrate the reader. If the calibration due date is missed,		
top of each screen.	The LED's on-time limit has been reached.	the reader can be used; however, the performance is not guaranteed.		
The following message displays: System out-of-order – Please contact Technical Support.	The reader is out of order.	Contact Technical Support.		

	EZ–Fluo™ Camera and Spot Counter		
The following message displays: No uEye camera present.			
The following message displays: Could not open camera! Check your connection.	The camera is not properly connected.	Verify that the main plug of the camera USB cable is connected to the USB port that was used during installation	
The following message displays: The camera is not connected.			
		Verify that the main plug of the camera USB cable is connected to the USB port used during the installation.	
		Close and restart the software.	
The following message displays: Transfer error.	The camera is not properly connected.	Verify that the second plug of the camera USB cable is connected to a USB port of the PC. If the problem persists, disconnect other peripheral devices connected to USB ports of the PC. Then close and restart the software.	
		Verify that the main plug of the camera USB cable is connected to the USB port used during the installation.	
	The camera is not properly connected.	Close and restart the software.	
The following message displays: An unspecified error has occurred.		Verify that the second plug of the camera USB cable is connected to a USB port of the PC. If the problem persists, disconnect other peripheral devices connected to USB ports of the PC. Then close and restart the software.	

# Troubleshooting

Error Message or Symptom	Possible Cause	Remedy
		Verify that the camera is correctly placed on the reader.
The following message displays:		Open and close the drawer to reactivate the membrane lighting.
Too bright! Check your reader.	The memorane is not correctly lit.	Verify that the second plug of the camera USB cable is connected to a USB port on the PC. If the problem persists, disconnect other peripheral devices connected to USB ports of the PC. Then close and restart the software.
		Verify that the camera is correctly placed on the reader.
		Verify that the reader is connected to power source.
Nothing to capture! Check your reader.	The membrane is not correctly lit.	Open and close the drawer to reactivate the membrane lighting.
		Verify that the second plug of the camera USB cable is connected to a USB port on the PC. If the problem persists, disconnect other peripheral devices connected to USB ports of the PC. Then close and restart the software.
	Incorrect exposure time.	Verify that the camera is correctly placed on the reader.
No image is displayed and the		Verify that the reader is connected to power source.
progress bar is blocked during an image capture.		Verify that the camera is correctly placed on the reader.
	The membrane is not correctly lit.	Verify that the reader is connected to power source.

Protocol				
The Staining Solution is leaking from the Petri-Pad™.	The wrong side of the Petri-Pad™ has been used.	Make sure to put the staining solution into the Petri-Pad™ base (the face with the engraved logo).		
	The Petri-Pad <sup>™</sup> was inverted before the membrane transfer.	Do not invert the Petri-Pad <sup>™</sup> before the membrane being transferred.		
Staining Solution droplets on the Petri-Pad™ lid inhibit the reading.	The staining solution has not been completely absorbed by the pad and a droplet has fallen on the Petri-Pad™ lid during the staining step.	Wipe the Petri-Pad™ lid in a controlled environmental area.		
During the reading step, presence of dark area(s) on the membrane cause some spots to be less well marked.	The volume of staining solution (1,7mL) applied to the pad was not sufficient to saturate the pad.	Add additional staining solution in small amounts until the pad is fully saturated.		
	Air bubble(s) formed during the incubation step.	Ensure that the membrane is distributed directly and uniformly on the medium to avoid the formation of wrinkles and air bubbles.		
	Air bubble(s) formed during the staining step.	Ensure that the membrane is distributed directly and uniformly on the medium to avoid the formation of wrinkles and air bubbles.		

If any of the above problems persists, contact Technical Support.

# **Ordering Information**

Description	Qty/Pk	Catalog No.		
EZ-Fluo™ System Kits				
EZ-Fluo <sup>™</sup> System standard kit including 1 reader, 1 camera and installation CD	1	EZFKIT001WW*		
EZ–Fluo™ Hardware				
EZ-Fluo™ reader	1	EZFREAD01WW*		
EZ-Fluo™ camera	1	EZFCAM001		
EZ-Fluo <sup>™</sup> Accessories				
EZ-Fluo <sup>™</sup> validation protocol, US letter format	1	EZFLLTVP1		
EZ-Fluo™ validation protocol, A4 format	1	EZFLA4VP1		
Microfil® funnel dispenser 100 ml	1	MIACFD101		
Microfil® funnel dispenser 250 ml	1	MIACFD201		
EZ-Pak® Membrane Dispenser	1	EZDISP001		
Microfil® 3 place manifold and support	1	MIAC03P01		
EZ-Stream™ Pump	1	EZSTREAM1		
Silicone Hose for EZ-Stream™ Pump Autoclavable Internal diameter: 9.5mm (3/8 in) Length: 5.0 m (196.8 in)	1	STREAMTUB		
PVC Hose for EZ-Stream <sup>™</sup> Pump Not autoclavable Internal diameter: 9.5mm (3/8 in) Length: 3.0 m (118.1 in)	1	XX67000034		
Filter forceps	1	XX6200006		
Traceability labels Label Printing Software: available at www.millipore.com	1000	TRLABEL01		
Consumables				
EZ-Fluo™ Reagent Kit	57 tests	EZFREAG57		
100 ml Microfil® funnel and 0,45 $\mu m$ 47 mm black gridded S-Pak® Filters	150	MIHABG100		
100 ml Microfil® funnel and 0,45 $\mu m$ 47 mm white gridded S-Pak® Filters	150	MIHAWG100		
250 ml Microfil® funnel and 0,45 $\mu m$ 47 mm black gridded S-Pak® Filters	150	MIHABG250		
250 ml Microfil® funnel and 0,45 $\mu m$ 47 mm white gridded S-Pak® Filters	150	MIHAWG250		
100 ml Microfil® funnel and 0,45 $\mu m$ 47 mm black gridded EZ-Pak® Filters	150	MZHABG101		
100 ml Microfil® funnel and 0,45 $\mu m$ 47 mm white gridded EZ-Pak® Filters	150	MZHAWG101		
250 ml Microfil® funnel and 0,45 $\mu m$ 47 mm black gridded EZ-Pak® Filters	150	MZHABG251		
250 ml Microfil® funnel and 0,45 $\mu m$ 47 mm white gridded EZ-Pak® Filters	150	MZHAWG251		
EZ-Pak® Filters 0.45 $\mu m$ 47 mm black gridded	600	MSP000814		
EZ-Pak® Filters 0.45 μm 47 mm white gridded	600	EZHAWG474		
S-Pak® Filters 0.45 μm 47 mm black gridded	600	HABG047S6		
S-Pak® Filters 0.45 µm 47 mm white gridded	600	HAWG047S6		
Petri-Pad <sup>™</sup> Petri dish, sterile, 47 mm	150	PD20047S0		
Petri-Pad™ Petri dish, sterile, 47 mm	600	PD20047S5		

\*Country code to be defined at ordering step

# Standard Product Warranty

The applicable warranty for the products listed in this publication may be found at: www.millipore.com/ec/cp3/terms (within the "Terms and Conditions of Sale" applicable to your purchase transaction).

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

### For more information:

Visit http://www.millipore.com/techservice or, in the U.S., call 1-800-MILLIPORE (1-800-645-5476).



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