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bCUBE 2.0 Quick Start Guide

Hyris Platform guide

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Index

Hyris bCUBE 2.0
Box Opening4
Device Overview5
Status LEDs
First set up
PC settings8
bCUBE 2.0 Connection
Interacting bCUBE 2.0: bAPP and bPANEL11
bAPP interface11
Access the bAPP11
Create a New Analysis: general information12
Create a new analysis: the cartridge14
Launch analysis on bCUBE16
Analysis details16
bPANEL 2.0 interface20
Installation
Main view21
Settings: Log in/Sync and bCUBE 2.0 connection21
New analysis creation22
Run the analysis23
Old analyses view24
Flow Chart25
Appendix-1: Set bCUBE 2.0 Wi-Fi from bCUBEs menu26
Appendix-2: Custom Recipe
Appendix-3: Cartridge Preparation
Load samples28
Load/Unload the cartridge to/from bCUBE 2.028



Hyris bCUBE 2.0

Box Opening

The following items will be found in the provided box:

- o **bCUBE 2.0** (Figure 1a)
- Ethernet cable (Figure 1b)
- Power Supply including European and North American power cords (Figure 1c). Characteristics:
 - Input: 100 ~ 240 V c.a. 1.4A 50/60Hz
 - Output: 12V DC 5A
 - **Power**: 60W
- **USB** stick with Hyris bPANEL software installer and User Manuals (Figure 1d)



Note: Please check the presence of all these items. Remove the items carefully from the shipping box and inspect them for any external damage. If any of the parts is missing or damaged, contact the manufacturer prior to installation.



Device Overview







Description of the features highlighted in the pictures above:

- Access Port Slider: opened to insert/remove the cartridge inside **bCUBE 2.0**. To be kept closed during operations.
- **Access port:** insert the cartridge here. Always push the cartridge up to the end of the port.
- **Thermal Block:** precisely controls the temperature of the cartridge and contained samples. Heating/Cooling is obtained with a Peltier element.
- Detection block: features high brightness LEDs with dichroic filters for fluorophores excitation. Detection is obtained thanks to a CMOS sensor with dichroic filters matched to fluorophores emission wavelengths.
- **Top venting holes:** allows air exchange between the inside of the **Instrument** and the ambient
- **Power supply Plug:** insert here the 12 VDC adapter jack
- **Power-on/Power-off button:** press shortly to wake up the instrument from Stand-By. When the machine is running, long press (about 5 seconds) to safely power down the **bCUBE**
- Ethernet Plug: insert here the Ethernet cable for Windows PC / Network communication
- Status LEDs: Give information about the status of the instrument

Status LEDs

The status of the **bCUBE** and its functioning are communicated to the user by the different colour combinations of the **Status LEDs** on the top of the device.

• **bDATA LED**: corresponding to the symbol, it indicates the connection status to Hyris server.

Ready colour: green

When Ready colour is on, **bCUBE 2.0** is connected to **bDATA**



• Thermal LED: corresponding to the 🖾 symbol, it indicates if the cartridge is hot or not. Ready colour: orange 🗕

When Ready colour is on, the internal temperature of the **bCUBE 2.0** is not hot. If cartridge is inserted, it can be safely handled.

• **Connection LED**: corresponding to the symbol, it indicates the internet connection state of the **bCUBE 2.0**

Ready colour: blue

When ready colour is on, **bCUBE 2.0** is connected to a network



For a graphical representation, the symbol (from left to right: **bDATA**, **Thermal** and **Connection LEDs**) will be displayed in this document when colour code explanation is required.

Some examples are reported in the following table:

LEDs Colours	DESCRIPTION	INTERPRETATION
	 bDATA LED steady green Thermal LED steady orange: Connection LED steady blue: 	 bDATA connection OK Cartridge not hot Network connection OK
	 bDATA LED steady green Thermal LED blinking orange and red Connection LED steady blue 	 bDATA connection OK WARNING: Cartridge hot Network connection OK



	 bDATA LED blinking green and yellow Thermal LED steady orange Connection LED blinking red and yellow 	 Missing bDATA connection Cartridge not hot Missing network connection
	 bDATA LED blinking green and yellow Thermal LED steady orange Connection LED steady blue 	 Missing bDATA connection Cartridge not hot Network connection OK. bCUBE 2.0 can be used locally
•••	 bDATA LED multicolour Thermal LED multicolour Connection LED multicolour 	- Experiment is running on bCUBE 2.0
•••	 bDATA LED off Thermal LED off Connection LED off 	- bCUBE 2.0 off

Note: not all the **Status LEDs** colour combinations are shown. For further information, please read the Owner's Manual.

First set up

PC settings

PC has to be connected to a stable internet connection.

bCUBE 2.0 Connection

In order to properly work, the following steps have to be respected:

- 1. Make sure the **bCUBE 2.0** is set on a flat and stable surface
- 2. Make sure to have correctly provided the condition explained in the PC Set-up section
- 3. Connect one end of the Ethernet cable to the **bCUBE 2.0** and the other to the PC according the following configuration





- 4. **Power** on the PC. Wait until PC is ready
- 5. Connect the PC to internet. Make sure internet connection is stable
- 6. Power on the **bCUBE 2.0**. Status LEDs colours: ------
- 7. bCUBE will start booting. Status LEDs colours: 🗸 🌈 🥠
- 9. Access the bAPP (see the <u>Access the bAPP</u> section) and go to the bCUBEs section. Select the desired bCUBE by checking the Serial number of the machine (see the Owner's manual). The "Connected" flag should be a green icon, indicating that the selected bCUBE is connected to a network. An action menu should be shown

item selected. CLEAR	Ą		C	÷	•	•		Ŧ
----------------------	---	--	---	---	---	---	--	---

10. Click in the solution to access the "Local settings page", then go to the "Network" section. Select one of the "Available WIFI Networks" and enter the related password, then click on

^{OK} . See the Owner's manual to perform **WPS** connection to the router or to manually add a network. The saved Wi-Fi should immediately appear in the "**Saved WIFI Networks**" column.



Network Status	Saved WiFi Networks		Available WiFi Networks
Current network WiFi[Example - Wifi]	🔶 Example - Wifi	Û	🔶 Example - Wifi - 3 +
IP address XXX.XXX.XXXXX	🗢 Example - Wifi - 2	Î	ᅙ Example - Wifi - 4 +
Hostname XX-XXXXXXXXXXXXX			🔶 Example - Wifi - 5 +
			ᅙ Example - Wifi - 6 +
			ADD MANUALLY WPS

11. When all the desired Wi-Fi networks are saved, go back to the **bAPP** and unplug the Ethernet cable from the **bCUBE**. After some seconds, the **Status LEDs** should show the Hyris Ready colours
. If not, check the troubleshooting section on the Owner's manual.

Note: Status LEDs may display different colours while **bCUBE 2.0** is looking for network connection. If no connection is detected, the following LEDs colours may be displayed after a minute of waiting time:



Interacting bCUBE 2.0: bAPP and bPANEL

bCUBE 2.0 can be controlled and monitored with two Hyris tools: **bAPP** and **bPANEL**.

- bAPP: it's a web app software and there's no need to install it on the PC. Ready-to-use recipes or custom recipes (see dedicated section) can be used to create and run analyses on the available devices according to the account permissions. Account and devices management are possible by this application. It can be run on PC or Smartphone. Internet connection is mandatory.
- bPANEL: this software interface is designed for offline usage of the bCUBE device, while also providing internet data sharing when connection is available. bPANEL 2.0 can start new analyses by using the already existing recipes (created on bAPP).

bAPP interface

System Requirements

РС

- Minimum computer requirements are:
- Intel Core i3 or equivalent, 4 GB RAM, Windows 7 or later, Ethernet or Wi-Fi connection.
- Windows PC minimum requirements: Windows 7, Windows 8, Windows 8.1, Windows 10 or later, Intel Pentium 4 processor or later that's SSE2 capable
- Mac minimum requirements: OS X Yosemite 10.10 or later
- Linux PC minimum requirements: 64-bit Ubuntu 14.04+, Debian 8+, openSUSE 13.3+, or Fedora Linux 24+ with Intel Pentium 4 processor or later that's SSE2 capable

Mobile

Minimum phone requirements are:

- Android 5.0 (Lollipop) or later, or iOs 12.0 or later

bAPP has been entirely developed on **Google Chrome** browser on for both PC or mobile. For this reason, **Google Chrome** is the only tested browser:

- Minimum version required for Google Chrome browser: 84.0.4147.89 or later

Access the bAPP

1. Make sure to have properly followed the instruction described in the <u>bCUBE 2.0 Connection</u> section.

If **bCUBE 2.0** is ready for operations, open the internet browser (Google Chrome browser recommended) on the PC and type <u>https://bapp.hyris.net</u> in the address bar. Then press enter to access the **bAPP**.

- 2. The Home window will be shown. The screen is mainly divided in two sections:
 - Main Menu: list of available bAPP sections:
 - **Home**: Welcome Window. Log-in credentials required when starting a new session on **bAPP**



- Analyses: all the available analyses are listed in this section. New analyses can be created
- **bKITs**: bKITs are reagent sets used to analyse a specific genetic sequence. The bKITs developed and sold by Hyris Ltd are listed in this section
- Recipes: a "Recipe" is a detailed list of steps and settings that the bCUBE has to execute while performing an analysis. New recipes can be customized from this menu (see <u>Appendix-2: Custom Recipe</u>). All the available recipes are listed here
- **Swarms**: "Swarm" is used to define a collector of analyses and recipes. User can have access to multiple swarms according to his permissions. Visible Swarms are listed in this section
- **Users**: list of available users and relative permissions (see Owner's Guide for further information)
- **bCUBEs**: list of available **bCUBEs** with relative extra information (see Owner's Guide for further information)
- **Certificates**: list of requested certificates.
- **Tab View Area**: content is related to the selected Main Menu section

Main Menu	Tab View Area
Hyris bApp ^{v1.5.0} Login	
🔒 Login	Ernal
HYRIS	Pesseed Remember me LOGIN SIGN UP FORGOT PASSWORD?

3. After the Main Window is displayed, **Login** is required.

Insert your email and password, which are corresponding to the **credentials** used to register the user to the Hyris Ltd web site or the **bAPP**. Enable the *"Remember me"* option to keep the user logged in.

In order to sign up, push the SIGN UP button and follow the steps.

In case of forgotten password, press on FORGOT PASSWORD? and insert the user mail. Then check your mail box for further instructions.

Create a New Analysis: general information



Go to the "Analyses" section from the Main Menu. The already existing analyses will be listed in a table.

In order to create a new analysis, click on the ⁺ button. The user will be guided through few steps to correctly fill all the requested information. In the **General Information** section, the user is asked to specify:

- o Name: name of the analysis
- **Description** (Optional): brief description of the experiment
- o Swarm: select the Swarm to which the analysis will be linked to
- **Recipe**: choose a recipe among the ones related to the selected Swarm.
- bKITs: show available bKITs (associated to the relative global recipe) instead of Swarm recipes

Click on to proceed to the cartridge set-up step.

1	General Informations	
	Name	Swarm
	Description	bKITs
		Recipe
	CONTINUE	

Custom recipe choice

In case of disabled "bKITs" option:

- 1. Select the desired **Swarm** among the list. In case only one available swarm for the user, it will be automatically selected. After the selection, the relative swarm recipes are loaded.
- 2. Select the desired **Recipe** among the list. Click on the **b** button to see the recap of the thermal protocol relative to the selected recipe.

bKIT choice

In the new/edit analysis page, after enabling bKITs [A], the following parts have to be filled:



1	General Informations		
	Name	Swarm	
	Description	bkits A	
		bKITs	3
		C D	þ
		E F	F

- A. **bKITs Flag**: if bKITs are enabled, fields [B], [C], [E] and [F] are shown
- B. **bKITs** categories (mandatory): the available bKITs depend on the selected category (examples: **Probiotics, Botanicals ...**)
- C. **bKIT part number** (mandatory): list of available bKITs according to the selected category. The bKIT part number is reported on the bKIT package as shown below.



Part Number: Bat**bKNuHp**terVERSION* Expiring Date

Note: Each **VERSION** of the bKIT is associated with a specific **Global Recipe**. Verify that the selected **bKIT** and its **Version** match the part number and version reported on the package.

- D. **QR Code Scanner**: if the desired bKIT is provided with dedicated QR code, click on this button and scan it. The QR code could be placed on the bKIT box and / or in the provided datasheet. In this case, the fields **[B]** and **[C]** are automatically set.
- E. **Global Recipe**: this is a read only field, which shows the name of the Global Recipe associated to the selected bKIT. It's automatically filled once **[C]** is selected
- F. **Datasheet/DNA extraction method download buttons**: click on the desired button to download a .pdf file of the desired document



1. Select the desired cartridge among the available ones (for example: "4x4 samples"). The wells information is filled while compiling the selected recipe.

Sample Sample ♥ ∎	Ð	Ð	e
÷	÷	÷	¢
Đ	÷	÷	e
Đ	÷	÷	Ģ

- 2. Wells can be deleted (press the ^{III} linked to the desired well), modified or added. In this last case:
 - a) Click on the desired well on the cartridge model on the right, according to the loaded sample
 - b) A dedicated window will appear
 - c) Select the Sample Type from the drop-down menu (Mandatory)
 - d) Enter the Sample Name (Mandatory)
 - e) Choose the Well Colour
 - f) Click on save button

Define well nr. 2		
Select well type		•
Name		
	CANCEL	SAVE
	UNITEL	0AVL

Other selection modalities are available for desktop mode:

• **Multiple selection of contiguous wells**: hold down the left button of the mouse. A cross cursor will appear. Holding down the button, drag the mouse on the cartridge to select

many contiguous wells. Click on the 🖍 button to open the "edit well" window.



Multiple selection of non-contiguous wells: hold down the CTRL button (a pointer cursor will appear) and click on the desired wells. Click on the
 button to open the "edit well" window.

You can then add a new well by repeating this procedure or click on

Launch analysis on bCUBE

If the "Launch now" option is selected, a new window will open. Select the desired bCUBE among the available devices. The selected bCUBE Status LEDs will blink. You can make the Status LEDs

blinking again by clicking on the 🚵 button. A recap of the thermal protocol that will be executed is

visible by clicking the ¹ button. *Cartridge Loading Wizard* can also be shown, and cartridge .pdf

file can be downloaded. Click on to immediately launch the analysis. The live analysis view page will be shown.

Run Analysis 🛛	POF	 ×
Select bCube		v

The **Status LEDs** of the device will start to show multiple and changing colours \frown \frown \frown \frown \frown Otherwise, check the Owner's Manual for further information.

If the "Save for later" option is selected, the user will be brought back to the Analyses list view.

Analysis details

GENERAL tab

This section shows the cartridge temperature measured by the instrument during the experiment and some information about the operating **bCUBE 2.0** (for example, its name and software version) or the analysis.

Each of these parameters are updated in real time while analysis is running and **bCUBE 2.0** is connected to the internet.

If the analysis state is "Running", the user can send commands to the operating bCUBE 2.0:

- ABORT : the running analysis will be immediately stopped
- EXTEND (5 ••••) : the extend button will add 5 cycles/minutes to the current step. This operation is not allowed when the "Melting Curve VAL" step is running.



• **SKIP** : this command instantly ends the current step in order to start with the following one.

Each of the previous actions will be tracked both in the analysis report and in the step preview on the **Tab View Area**.



WARNING

Make sure the previous command has been executed by **bCUBE 2.0** before sending a new command.

STEPS tab

All analysis steps are listed in this tab.

Each step data can be consulted individually by clicking on the correspondent name on the list. The step view will be expanded, showing extra data, such as the fluorescence/time graphic, which is updated in real time while the analysis is "Running" and **bCUBE 2.0** is connected to the internet.



If the analysis is completed, a computed parameter table will be added next to the graphic (if specified in the recipe).



Select parameter: TTS							
Q Search Table							
Sample	Туре	Channel	Value				
Positive control	PosCtrl	FAM	0:00:56				
Unknown 1	Sample	FAM	0:00:55				
Unknown 2	Sample	FAM	0:00:55				
Negative control	NegCtrl	FAM					
Unknown 3	Sample	FAM	0:01:08				
Unknown 4	Sample	FAM	0:01:07				
Unknown 5	Sample	FAM	0:01:08				
Unknown 6	Sample	FAM					

RESULTS Tab

In this tab a graphic representation of the selected cartridge is shown. After the analysis is completed, each sample is marked with a label indicating the result of the experiment (if specified in the recipe).





Action button

Click on the **i** button in the tab section to open the action menu. Different actions are available depending on the analysis status:

PDF

 Generate PDF button will immediately start the download of the report (.pdf file) of the current analysis

• Generate .XLSX button will download an Excel readable file with all the analysis information and results.

 Edit Samples button allows the user to change some wells information, such as the wells names or types.

도

• Recalculate results allows the user to change some settings of each step and to recompute the analysis results accordingly.

<>

• EXTEND the extend button will add 5 cycles/minutes to the current step. This operation is not allowed when the "Melting Curve - VAL" step is running.

×

 $\circ \quad \mbox{\sc ABORT} \quad \mbox{button: the running analysis will be immediately stopped}$

ÞI

• SKIP button: this command instantly ends the current step in order to start with the following one.



bPANEL 2.0 interface

System Requirements

In order to run **bPANEL** PC Interface, the minimum computer requirements are:

- Intel Core i3 or equivalent, 4 GB RAM, Windows 7 or later, Ethernet or Wi-Fi connection.



WARNING

Although **bCUBE 2.0** can work offline with **bPANEL** software, its connection to internet is required at least once a week. If not connected for longer periods of time, any action on vice will be blocked until new connection

the device will be blocked until new connection.

Installation

Open the bPANEL 2.0 installer located in the provided USB.

- 1) Insert into an available USB port the Hyris USB Stick containing bPANEL installer.
- 2) Launch the file Setup.msi, located inside bPANEL folder.
- 3) On the welcome screen, click on Next.

🙀 Hyris bPANEL Setup	- 🗆 X
Ð	Welcome to the Hyris bPANEL Setup Wizard
	The Setup Wizard allows you to change the way Hyris bPANEL features are installed on your computer or to remove it from your computer. Click Next to continue or Cancel to exit the Setup Wizard.
	Back Next Cancel

Figure 1: bPANEL installation - Welcome screen

4) On the next page, carefully read the licence agreement. Tick the check-box and click **Install** to continue.



5) Wait for the installation process to end. Click on **Finish** on the last page to complete the installation.

No driver installation is required for using **bCUBE 2.0**.



Main view

Make sure all the steps described in <u>bCUBE 2.0 Connection</u> have been correctly performed. Open the bPANEL 2.0 program. The main view will be shown:

- Command Buttons: list of command to send to the connected bCUBE
- Tab selection: each tab provides different information about the loaded analysis or the user settings. The analysis related tabs are updated in real time while the selected analysis is running and the correspondent bCUBE is connected
- o Tab View Area: shows the content of the selected tab



Settings: Log in/Sync and bCUBE 2.0 connection Go to "Settings" tab:

1. Click on Login

bCube IP/Name		•	Clear/Refresh
bData Username:			
Last Comm			

 Insert your email and password, which are corresponding to the credentials used to register the user to the Hyris Ltd web site (same as used credential to access the bAPP. See <u>Access</u> <u>the bAPP</u> section for further information). Then click "OK"



Data Username: TestUser@demo.net			
Data Dascword	bData Username:	TestUser@demo.net	
	bData Password:	*******	

3. Click on Search . After the operation completion, the available **bCUBEs** devices will be listed in the dedicated drop-down menu. Select the desired one

bCube IP/Name	YYY.YYYY.YY.YY * Cearch
bCube Host Name	YY. YYYYYYYYYYY
	Testliser@demo.net

4. Click on the selected device. After connection, the button will turn green

New analysis creation

- 1. Click on the **G** button
- 2. Insert the Analysis Name (Mandatory)
- 3. Select the desired Swarm among the available ones (Mandatory)
- 4. Choose whether to search among Swarm recipes or Global recipes
- 5. Select the desired Recipe
- 6. Click on ^{Ok} button.

Analysis name:	DEMO_ANALYSIS	
Select Cartridge type:	16wells 👻	
Select Swarm:	Test	Ŧ
	O Swarm recipes	
Select Recipe:	Panax spp. 1.1	~



7. The new analysis will be created and tabs updated accordingly.

The default wells information are defined in the selected recipe (downloaded from **bDATA**).

Protocol	Samples		🗲 Settings								
_ 1 Sample	· · ·	■▼	INSERT SAMPLE NAME	×	₽ 	INSERT SAMPLE NAME	~	₽	INSERT SAMPLE NAME	Ŷ	₽
INSERT SAMPLE NAMI	e v	0	INSERT SAMPLE NAME	Ŷ	0	INSERT SAMPLE NAME	v	\$	INSERT SAMPLE NAME	Ŷ	₽
INSERT SAMPLE NAMI	e v	0	INSERT SAMPLE NAME	v	0	INSERT SAMPLE NAME	Ŷ	>	INSERT SAMPLE NAME	Ŷ	₽
INSERT SAMPLE NAMI	e v	9	INSERT SAMPLE NAME	Ŷ	9	INSERT SAMPLE NAME	v	8	INSERT SAMPLE NAME	v	•

- 8. Wells can be deleted, modified or added. In this last case:
 - a. Click on the desired well on the cartridge model on the right, according to the loaded sample
 - b. Select the Sample Type from the drop-down menu (Mandatory)
 - c. Enter the Sample Name (Mandatory)
 - d. Choose the Sample Colour



Run the analysis

- 1. Load the cartridge to the selected **bCUBE 2.0** (see section: <u>Cartridge Preparation</u>).
- 2. Run the analysis by clicking on the command button. The analysis will be uploaded to the selected **bCUBE 2.0**. If no errors occur, the experiment will immediately start, while the

Status LEDs of the device will start to show multiple and changing colours $\frac{1}{2}$

- 3. The analysis parameters and results will be automatically shown in specific tabs in real time.
- 4. Wait for "Ready Colours" -
- 5. Generate a PDF report file by clicking on the command button, and then selecting "Build/Rebuild PDF Report".
- 6. Unload the cartridge



Old analyses view

Old analyses can be consulted by opening them from PC, **bCUBE** or online **bDATA** folders.

Click on the command button and select the desired folder location among "LOCAL", "bCUBE" and "bDATA". Click on "OK" after the analysis selection. The analysis data will be loaded to bPANEL and results can be consulted. The opened analysis can't be modified.



Flow Chart





Appendix-1: Set bCUBE 2.0 Wi-Fi from bCUBEs menu

If the **bDATA LED** is showing steady green colour, **bCUBE 2.0** Wi-Fi can also be configured from "**bCUBEs**" section of the **bAPP**.

- 1. Access the **bAPP** and Log In as shown in the <u>PC: bAPP Interface</u> section
- 2. Click on **"bCUBEs**" from the main menu: a list of the available devices will appear. The available devices could be different among users of the same company depending on their permissions.
- 3. Select the desired device and press the $\widehat{}$ button.
- 4. Select the desired WLAN and set the Wi-Fi password, then click on ADD

WiFI Setup WiFi(s) Discovered	
🔶 Example WiFi LAN	^
WiFi Password	



Appendix-2: Custom Recipe

New recipes can be customized on the **bAPP** according to user's permissions. If recipe customization is not allowed by user's permissions, global recipes provided by Hyris Ltd or already existing company recipes can be used for new analyses.

1. Click on "Recipes" in the left side Main Menu: the list of the available recipes will be shown.

Recip	bes				+	C	Columns 🔻
٩	Search						All Columns 🔻
	↑ Date	t₁ Name	↑₊ Swarm	↑, Description			
\bigcirc	2019-09-18 15:26	ExampleRecipe	Test				

- 2. Click on the + button to access to the new recipe customization form. Remember that **Recipe name** and selected **Swarm** are mandatory.
- 3. Follow the steps and click on FINISH button.
- 4. The new recipe will be added to the recipe list



Appendix-3: Cartridge Preparation

Load samples

In order to load the samples for the experiment:

- 1. Place the cartridge face up on a flat and stable surface
- 2. Put the sample in one or more of the cartridge wells as indicated in the reference Recipe.
- 3. Seal the cartridge with the provided aluminium foils





WARNING

Never flip the cartridge upside down or directly touch the wells.

Load/Unload the cartridge to/from bCUBE 2.0

To load the cartridge to the **bCUBE 2.0**:

- 1. Open the **bCUBE 2.0** Access Port slider
- 2. Insert the cartridge by pushing it through the Access Port by keeping it face up holding it from the handle till it stops
- 3. Close the access port slider

To unload the cartridge:

- Make sure the cartridge is not hot as indicated by the Steady orange colour of the Thermal LED .
- 2. Open the access port slider
- 3. Gently pull the cartridge from the handle
- 4. Close the access port slider