ICELL8® cx Single-Cell System User Manual

Cat. Nos. 640188, 640189, 640187, 640002, 640198
(042519)
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I. Introduction

A. Thank You for Your Order!
Congratulations on the purchase of your ICELL8 cx Single-Cell System, referred to as ICELL8 cx in this manual. The ICELL8 cx is designed to load samples into chips and then perform imaging.

B. About this Manual
This manual provides instructions for the safe operation and maintenance of the ICELL8 cx:

Symbols and Conventions
The following symbols and conventions (Table I) are used throughout this manual.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>![ Icon ]</td>
<td>DANGER: Indicates a hazardous situation that could result in death or serious injury.</td>
</tr>
<tr>
<td>![ Icon ]</td>
<td>WARNING: Indicates a potentially hazardous situation that could result in injury to the user or damage to or destruction of the system.</td>
</tr>
<tr>
<td>![ Icon ]</td>
<td>CAUTION: Indicates a hazard that could result in loss of data or damage to the system.</td>
</tr>
<tr>
<td>![ Icon ]</td>
<td>Indicates the presence of an electrical shock hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION symbol.</td>
</tr>
<tr>
<td>![ Icon ]</td>
<td>Indicates the presence of a biological hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION symbol.</td>
</tr>
<tr>
<td>![ Icon ]</td>
<td>Indicates the presence of a mechanical or pinch hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION symbol.</td>
</tr>
<tr>
<td>![ Icon ]</td>
<td>IMPORTANT: Provides information on proper system operation.</td>
</tr>
<tr>
<td>![ Icon ]</td>
<td>NOTE: Provides helpful ancillary information to support the use of the system.</td>
</tr>
</tbody>
</table>

C. Technical Support
Review the information in this manual thoroughly before starting your reactions. Also review documentation supplied with the accessory equipment you are using. If you require technical support, you can contact your authorized TBUSA service technician or TBUSA directly at technical_support@takarabio.com.
D. **ICELL8 cx Safety Information**

**CAUTION:** There are no user-serviceable parts inside the instrument. Service of any internal parts should be performed by a qualified TBUSA service technician.

**Operating conditions:**

The instrument is safe to operate with the covers in place. The covers protect the user from moving parts and must not be removed during operation. If this equipment is not used as specified by the manufacturer, the protection provided by this equipment may be impaired.

Operate the ICELL8 cx only inside an appropriate building. Do not operate the ICELL8 cx outside or in wet environments.

**Instrument use:**

**WARNING:** Use of the ICELL8 cx instrument requires users to wear appropriate personal protective equipment (PPE) which should, at minimum, include gloves, eye protection, and a lab coat. However, the choice of PPE used should be dictated by the biosafety level of the biological samples being introduced into the ICELL8 cx system. Please consult your institutional biosafety committee for additional information on the necessary precautions for your sample type.

**WARNING:** Class I Equipment: This equipment must be grounded. The power plug must be connected to a properly wired grounded outlet. An improperly wired outlet could place hazardous voltages on accessible metal parts.

**CAUTION:** Do not position the equipment so that it is difficult to operate the power switch or remove the power cord.

**WARNING:** Use only the power cord provided by the manufacturer. Do not replace the power cord with an inadequately rated cord.

**WARNING:** The machine is powered by two cords. The instrument is off only when the power switch is off and the ethernet power cord is disconnected. See image below.

---

Figure 1. The ICELL8 cx instrument.
Certification and standards information:


Safety specifications are also met under the following environmental conditions, which are in addition to those stated in the operating conditions:

- **Installation Category (overvoltage category) II according to IEC 60664-1.** The Installation Category defines the level of transient overvoltage which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its means of overvoltage protection. For example, in CAT II, which is the category typically used for instruments in hospital, research, and industrial laboratories, the expected transient overvoltage is 2,500 V for a 230-V supply and 1,500 V for a 120-V supply.

- **Pollution Degree 2 according to IEC 60664-1.** Pollution Degree 2 assumes that normally only nonconductive pollution (e.g., dust) are present in the operating environment, with the exception of occasional conductivity caused by condensation.

Both the Installation Category (overvoltage category) and the Pollution Degree affect the dimensioning of electrical insulation within the instrument.

Moving the system:

**WARNING:** If you need to move the system after it has been installed, please contact Takara Biosystems, Inc.

Warning labels on the instrument:

Please note the warning label on the instrument.

**WARNING:** This system contains moving parts. Keep hands away from the system while the instrument is in use.
## II. System Description: Component Overview

The complete ICELL8 cx Single-Cell System (Cat. No. 640188, 640189*) includes the components listed below, and ICELL8 cx CellSelect® Software (Cat. No. 640198).

<table>
<thead>
<tr>
<th>ICELL8 cx Single-Cell System (Cat. No. 640188, 640189*)</th>
<th>Quantity per kit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ICELL8 cx Instrument</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>ICELL8 cx Thermal Cycler</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Regional Power Cord</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>Universal Power Strip</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>ICELL8 cx Accessory Kit</strong></td>
<td>1</td>
</tr>
<tr>
<td>2 x ICELL8 cx Centrifuge Chip Spinner</td>
<td></td>
</tr>
<tr>
<td>1 x ICELL8 cx Centrifuge Balance</td>
<td></td>
</tr>
<tr>
<td>1 x ICELL8 cx Cold Block</td>
<td></td>
</tr>
<tr>
<td>1 x Nanodispenser Blotter</td>
<td></td>
</tr>
<tr>
<td>2 x ICELL8 Chip Holder</td>
<td></td>
</tr>
<tr>
<td>1 x Magnetic Tube Stand</td>
<td></td>
</tr>
<tr>
<td>1 x Plate Seal Applicator</td>
<td></td>
</tr>
<tr>
<td><strong>ICELL8 cx Installation Kit</strong></td>
<td>1</td>
</tr>
<tr>
<td>1 x Imitation Master Mix with UV Dye &amp; Rox (45 ml)</td>
<td></td>
</tr>
<tr>
<td>1 x Nanodispenser Alignment Chip</td>
<td></td>
</tr>
<tr>
<td>1 x Nanodispenser Chip Alignment Film (Pack of 10)</td>
<td></td>
</tr>
<tr>
<td>1 x Blotting Paper (Pack of 10)</td>
<td></td>
</tr>
<tr>
<td>3 x 384-Well Source Plate Seal</td>
<td></td>
</tr>
<tr>
<td>3 x 384-Well Source Plate</td>
<td></td>
</tr>
<tr>
<td>2 x Chip Balance</td>
<td></td>
</tr>
<tr>
<td>1 x Handheld Magnifier with UV Illumination</td>
<td></td>
</tr>
<tr>
<td>2 x ICELL8 Extraction Fixture – L</td>
<td></td>
</tr>
<tr>
<td><strong>ICELL8 cx Loading Kit</strong></td>
<td>3</td>
</tr>
<tr>
<td>2 x Blotting Paper</td>
<td></td>
</tr>
<tr>
<td>1 x Optical Imaging Film</td>
<td></td>
</tr>
<tr>
<td>1 x TE Sealing Film</td>
<td></td>
</tr>
<tr>
<td>1 x SmartChip Intermediate Film</td>
<td></td>
</tr>
<tr>
<td><strong>ICELL8 Collection Kit – L</strong></td>
<td>3</td>
</tr>
<tr>
<td>1 x Collection Fixture – L</td>
<td></td>
</tr>
<tr>
<td>2 x Collection Tube (2.0 ml)</td>
<td></td>
</tr>
<tr>
<td>1 x Collection Film</td>
<td></td>
</tr>
<tr>
<td><strong>ICELL8 384-Well Source Plate and Seal</strong></td>
<td>1</td>
</tr>
<tr>
<td>5 x 384-Well Source Plate</td>
<td></td>
</tr>
<tr>
<td>5 x 384-Well Source Plate Seal</td>
<td></td>
</tr>
<tr>
<td><strong>ICELL8 cx Blank Chip - 250 nl FB</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>ICELL8 Blank Chip Reagent Kit</strong></td>
<td>3</td>
</tr>
<tr>
<td>2 x 1X ICELL8 Fiducial Mix (25 µl)</td>
<td></td>
</tr>
<tr>
<td>2 x 100X Second Diluent (15 µl)</td>
<td></td>
</tr>
<tr>
<td><strong>ICELL8 cx 3’ DE Chip</strong></td>
<td>2</td>
</tr>
<tr>
<td><strong>ICELL8 3’ DE Reagent Kit</strong></td>
<td>2</td>
</tr>
<tr>
<td>5 µl Control K-562 RNA (1 µg/µl)</td>
<td></td>
</tr>
<tr>
<td>5 µl MgCl2 (1 M)</td>
<td></td>
</tr>
</tbody>
</table>
III. System Description: Instrument Overview

The ICELL8 cx instrument includes the following main components:

A. Desktop computer and monitor
B. Water reservoir and electronic scale
C. Wash bottle
D. Humidifier (not visible) and humidifier reservoir
E. Waste container

Figure 2. The ICELL8 cx instrument (external view). This image is for Cat. No. 640188. Note: The thermal cycler is not included in Cat. No. 640189.

*640189 is the ICELL8 cx Single-Cell System for Japan. It does not include a Thermal Cycler, and therefore only includes 4 power cords.
**This component is also sold separately.
Behind the front access doors are the following components:

F. Degassers  
G. Syringe pump bank  
H. Environmental chamber  
I. cx stage module  
J. XY transitional axes  
K. Tip nozzle assembly & optics module  
L. Chip nest (left) & 384-well plate nest (right)  
M. Trough  

Water reservoir and electronic scale

The water reservoir contains deionized Milli-Q water (or equivalent) that occupies all fluid paths in the ICELL8 cx instrument. The liquid is used to draw reagents into and push out of the tips. The water reservoir sits on an electronic scale that monitors water level, so that users can make sure there is enough water prior to starting a chip-dispense operation.
Wash bottle
The Wash bottle is the source for the Wash well in the trough. It is to be filled with a 0.2% sodium hypochlorite solution, which is used for cleaning the tip nozzles after dispenses to prevent cross-contamination.

Humidifier and humidifier reservoir
The Humidifier is used to maintain the relative humidity levels in the environmental chamber to minimize reagent evaporation during the dispensing process. A sensor embedded in the platform base is used to monitor the enclosure temperature, relative humidity, and chip temperature. The system adjusts the humidity and chip temperature so that the chip is at or near the dew point temperature, which minimizes evaporation during sample dispensing. The Humidifier is behind the back cover of the ICELL8 cx unit (Figure 5, below).

Figure 5. Humidifier unit. The dotted-line box indicates the approximate location of the humidifier assembly under the back cover.

Degassers
The degassers sit to the left of the syringe pump bank. Their purpose is to remove dissolved gas from the system fluid, helping the system maintain good dispense quality.

Syringe pump bank
The syringe pump bank consists of eight hydro-pneumatic systems that drive the aspiration and dispensing of samples and reagents in the ICELL8 cx instrument. A system of tubing connects the tip nozzle assembly and the syringe pumps. The syringe pumps are used in tandem with solenoid valves to generate discrete droplets during a dispense sequence.

Waste container
The waste container collects the fluid that is discarded through the tip nozzles into the rinse and waste wells of the trough.
Stage module

The stage module houses the XY transitional axes, tip nozzle assembly and optics module, chip and source plate nests, and trough. The XY transitional axes transport the tip nozzle and optics module around the stage. When the door to the stage module is closed, it creates an environmental chamber that encompasses the stage module to maintain optimal humidity levels during sample and reagent dispensing.

Prior to any dispense or scan, the optics module confirms the correct orientation of the chip and reads the chip barcode. Samples are aspirated from a 384-well plate that sits in the source plate nest and are dispensed into a chip that sits in the chip nest. The tips are brought to the trough to discard excess fluids to waste and for cleaning. After a sample is dispensed, the optics module scans the chip to generate a map (a.k.a. filter file) of the wells that contain single cells. The dispensing process is repeated for all downstream reagents, but they will only be dispensed to the wells that are flagged in the filter file.

Figure 7. ICELL8 cx stage module. The environmental chamber refers to the stage module area and the chassis enclosing it.

Trough

The trough consists of three wells. From left to right they are the “Rinse”, “Waste”, and “Wash” wells. The rinse well is used for rinsing the inside and outside of the tip with system water and drains to the waste container. The waste well is also connected to the waste container and is where any excess system fluid, reagents, or samples are discarded. The wash well is connected to the wash bottle (which contains 0.2% sodium hypochlorite solution) and used for washing the inside and outside of the tips with the solution.

Figure 8. The trough component.
A. ICELL8 cx Specifications and Lab Requirements

Table II. ICELL8 cx specifications and lab requirements.

<table>
<thead>
<tr>
<th>Category</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispense volume</td>
<td>50 nl, or 100 nl per nanowell</td>
</tr>
<tr>
<td>Software</td>
<td>ICELL8 cx Software (pre-installed)</td>
</tr>
<tr>
<td>Computer</td>
<td>Enterprise Level Windows 10 PC (included with system)</td>
</tr>
<tr>
<td>Power requirements (for different electrical grid types)</td>
<td>120 VAC/60 Hz mains: four 120 V, 15 or 20A circuits</td>
</tr>
<tr>
<td></td>
<td>220–240 VAC/50 Hz mains: four 10A outlets</td>
</tr>
<tr>
<td></td>
<td>100 VAC/50–60 Hz mains: four 15A outlets</td>
</tr>
<tr>
<td>Fuses</td>
<td>Dispenser; 4 Ah, 250 V</td>
</tr>
<tr>
<td>Environmental conditions</td>
<td>Ambient temperature: 15–30°C</td>
</tr>
<tr>
<td></td>
<td>Relative humidity, non-condensing: 30–70%</td>
</tr>
<tr>
<td></td>
<td>Altitude: &lt;2,000 m from sea level</td>
</tr>
<tr>
<td></td>
<td>Pollution degree: 2 or less</td>
</tr>
<tr>
<td>Dimensions</td>
<td>Desktop Computer Tower: 7” W x 14.5” H x 17.5” D (18 cm x 37 cm x 44 cm)</td>
</tr>
<tr>
<td></td>
<td>ICELL8 cx Module: 42.5” W x 23” H x 23” D (108 cm x 58 cm x 58 cm)</td>
</tr>
<tr>
<td>Bench space</td>
<td>Bench space including clearance for ICELL8 cx Module and Desktop Computer: 70” W (minimum) x 38” H (minimum) x 30” D (178 cm x 97 cm x 76 cm)</td>
</tr>
<tr>
<td></td>
<td>*Note: Bench space must be capable of supporting 200 pounds (91 kg)</td>
</tr>
<tr>
<td>Floor space</td>
<td>Waste Container: 10” W x 10” H x 10” D (25 cm x 25 cm x 25 cm)</td>
</tr>
<tr>
<td>Weight</td>
<td>200 pounds (91 kg)</td>
</tr>
</tbody>
</table>

B. Setup and Installation

Your TBUSA Service Engineer will unpack and install your ICELL8 cx and explain the basic operation of the system. They will use material from the ICELL8 cx Installation Kit to qualify the instrument after installation and will leave reusable and/or remaining materials at your site. Table III (below) lists the ICELL8 cx Installation Kit components.

Table III. ICELL8 cx Installation Kit components.

<table>
<thead>
<tr>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanodispenser Alignment Chip</td>
</tr>
<tr>
<td>Nanodispenser Chip Alignment Film</td>
</tr>
<tr>
<td>Imitation Master Mix with UV Dye &amp; Rox (45 ml)</td>
</tr>
<tr>
<td>Blotting Paper (Pack of 10)</td>
</tr>
<tr>
<td>384-Well Source Plate (3)</td>
</tr>
<tr>
<td>384-Well Source Plate Seal (3)</td>
</tr>
<tr>
<td>Chip Balance (2)</td>
</tr>
<tr>
<td>Handheld Magnifier with UV Illumination</td>
</tr>
<tr>
<td>ICELL8 Extraction Fixture – L (2)</td>
</tr>
</tbody>
</table>

**NOTE:** To avoid contaminating your PCR, do not install the ICELL8 cx instrument in an area that could contain high-copy DNA or amplicons from previous PCR experiments.
C. Required Reagents and General Lab Equipment

Reagents for ICELL8 cx Reservoirs
- Deionized filtered water (Milli-Q or Elga system or equivalent; 0.2-µm filtration).
- 0.2% sodium hypochlorite solution made from reagent-grade sodium hypochlorite in deionized filtered water.

Other Reagents and Materials
- 70% isopropanol or equivalent (for example, 70% Reagent Grade Alcohol [VWR, Cat. No. 89370-078]) for priming the system tubing.
- Refer to application-specific user manuals for details about required samples and reagents.

General Lab Equipment
- Secondary containers (e.g. 1L or 500ml bottles) for refilling reservoirs and for alcohol priming.
- Kimwipes or similar dry wipes for cleaning the stage module.
- Refer to application-specific user manuals for any other required equipment.

IV. System Description: SmartChip Technology

SmartChip technology distinguishes TBUSA’s platform from other systems. Each chip has a 72 x 72 array of nanowells and can accommodate up to 5,184 reactions in a single run.

The ICELL8 cx unit transfers samples, controls, and reagents from the 384-well plate to the chip. After dispensing, the chip can be processed for cell analysis (Figure 9).

Figure 9. ICELL8 cx cell scan workflow.
V. Protocol: Quick Reference Guide

Table V below is a quick reference guide for the general workflow of dispensing samples into the chip, scanning cells, and dispensing downstream reagents. Print this guide for easy reference in the laboratory. Each step is described in detail in the sections following the quick reference guide.

Table IV. Quick reference guide.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Prepare the ICELL8 cx System (Page 16)</td>
</tr>
<tr>
<td></td>
<td>a. Power on the system</td>
</tr>
<tr>
<td></td>
<td>b. Check and fill system containers</td>
</tr>
<tr>
<td></td>
<td>c. Run the Daily Warmup</td>
</tr>
<tr>
<td></td>
<td>d. Perform alcohol prime of system tubing (if needed)</td>
</tr>
<tr>
<td></td>
<td>e. Run the Tip Clean procedure</td>
</tr>
<tr>
<td>2.</td>
<td>Prepare the sample source plates (Page 22).</td>
</tr>
<tr>
<td></td>
<td>a. Pipette cell samples into a 384-well sample source plate</td>
</tr>
<tr>
<td></td>
<td>b. Seal the plate with imaging film; do not centrifuge</td>
</tr>
<tr>
<td>3.</td>
<td>Dispense samples into the chip (Page 22).</td>
</tr>
<tr>
<td></td>
<td>a. Select the ICELL8 cx application</td>
</tr>
<tr>
<td></td>
<td>b. Place the chip in the ICELL8 cx unit</td>
</tr>
<tr>
<td></td>
<td>c. Place the sample source plate in the ICELL8 cx unit</td>
</tr>
<tr>
<td></td>
<td>d. Dispense sample cells and controls into the chip</td>
</tr>
<tr>
<td>4.</td>
<td>Blot and centrifuge the chip (Page 28)</td>
</tr>
<tr>
<td></td>
<td>a. Blot the chip</td>
</tr>
<tr>
<td></td>
<td>b. Seal with application-appropriate film</td>
</tr>
<tr>
<td></td>
<td>c. Centrifuge the sealed chip</td>
</tr>
<tr>
<td>5.</td>
<td>Scan chip for single cells and freeze the chip (Page 28)</td>
</tr>
<tr>
<td></td>
<td>a. Scan the chip with the ICELL8 cx Imager</td>
</tr>
<tr>
<td></td>
<td>b. Save the results (a filter file will be generated)</td>
</tr>
<tr>
<td></td>
<td>c. Freeze the chip</td>
</tr>
<tr>
<td>6.</td>
<td>Dispense reagents into the chip (Page 31).</td>
</tr>
<tr>
<td></td>
<td>a. Place the chip and reagent source plate in the ICELL8 cx system</td>
</tr>
<tr>
<td></td>
<td>b. Select the filter file generated from the cell scan step</td>
</tr>
<tr>
<td></td>
<td>c. Dispense reagents into chip</td>
</tr>
<tr>
<td>7.</td>
<td>Run your PCR on a thermal cycler (Page 33).</td>
</tr>
<tr>
<td>8.</td>
<td>Clean the ICELL8 cx System (Page 34).</td>
</tr>
</tbody>
</table>
VI. Protocol: Prepare the ICELL8 cx System

A. Power on the System

IMPORTANT: Make sure that the ICELL8 cx Module is connected to the USB 3.0 ports on the computer (blue ports), and not the USB 2.0 ports (black ports). Connecting to the USB 2.0 port will result in software errors.

1. Power up the system by flipping the switch at the back-left of the ICELL8 cx unit.
2. Start the ICELL8 cx Software on the computer by double-clicking the “ICELL8 cx” icon on the desktop.

a. The XY axes, syringe pumps, and optics module will automatically initialize when the software is connected to the ICELL8 cx module.

b. After the system is powered up, it may take 10 minutes for it to stabilize. The live chamber temperature, chamber relative humidity, chuck setpoint, and chuck temperature are displayed in the boxes below. (Note: Temperature is reported in degrees Celsius.)

![Example of DewPoint parameter display.](image)

B. Check and fill system containers

Prior to dispensing, click on the Startup tab and follow the list to check the system containers.

![System startup menu in the cx instrument software.](image)
Figure 12. Items checked during the instrument startup process.

1. Check the amount of water in the water reservoir. Make sure the Water Bottle is filled with Milli-Q water with at least 800 ml. Refill as needed following these steps:
   a. Fill a clean secondary container with deionized filtered water.
   b. Carefully unscrew the cap of the water reservoir bottle.
   c. Slightly lift the cap up with one hand and use your other hand to pour water from the secondary container into the water reservoir bottle.

   Figure 13. Recommended method of refilling the water reservoir.

   d. After the bottle has been refilled, screw the water reservoir bottle cap back on.
2. Check the humidifier reservoir and refill with MilliQ water as needed.
   a. Use the same procedure for refilling the humidifier reservoir as described above for the water reservoir.

   ![Recommended method of refilling the humidifier reservoir](image)

   **Figure 14.** Recommended method of refilling the humidifier reservoir.

3. Check that the **waste container** is no more than half-full. If so, dispose of liquid waste appropriately and re-install the empty Waste container following these steps:
   a. To uninstall the Waste container, first locate the cap and press the quick-release buttons to release the tubings.
   b. Carefully set the tubings aside and transport the Waste container to the disposal location.
   c. Unscrew the cap and properly dispose of contents.
   d. Screw the cap back on and re-install container by plugging the tubing back into the port in the cap.

4. Check the **wash bottle** and replace sodium hypochlorite solution if volume is < 50% or if the solution is more than a week old. Follow these steps to replace the solution:
   a. Disconnect the quick-connect fitting at the top of the cap and set tubing aside on a clean surface.
   b. Remove cap from Wash bottle and place cap on a clean surface.
   c. Properly dispose of the old fluid and refill with 500ml of fresh 0.2% reagent grade sodium hypochlorite.
   d. Screw the cap of the Wash bottle back on and re-connect the quick-connect tubing.

   ![Disconnecting the quick-connect fitting from the wash bottle cap](image)

   **Figure 15.** Disconnecting the quick-connect fitting from the wash bottle cap.
e. Go to the Advanced tab and click [Wash Prime] to prime tubing with fresh 0.2% sodium hypochlorite solution.

![Image of Advanced tab with Wash Prime highlighted](image-url)

Figure 16. The [Wash Prime] button under the Advanced tab of the instrument software.

C. Run the Daily Warmup

**IMPORTANT:** Run the Daily Warmup each day prior to performing any experiments. Failure to do so may result in poor dispensing.

1. After the check of the containers is complete, click the [Done] button for each of the categories:

![Image of Daily checklist after completing all the check steps](image-url)

Figure 17. Daily checklist after completing all the check steps.

2. Click the [Start] button next to “Daily Warmup”. The warmup process takes roughly 5 min. During this time, the ICELL8 cx will do the following:
   a. Home the XY axes and initialize the syringe pump bank.
   b. Perform a System Prime to flush the system tubing with fresh water and purge trapped air.
   c. Perform a Tip Clean.

During the warmup, monitor the syringes for trapped bubbles. If bubbles are observed, repeat the “Daily Warmup” process up to two times.
NOTE: If large bubbles are still observed in syringes or tubing (Figure 11) after three warmup cycles, we recommend priming the system tubing with isopropyl alcohol. Continue to Section VI.D for the priming protocol. If there are no large bubbles observed in the syringes or tubing, skip to Section VI.E.

Figure 18. Example of large bubbles in syringes.

D. Perform alcohol prime of system tubing (if needed)

An alcohol prime of the system tubing should be performed once at the beginning of the work week and when air bubbles are observed to be trapped in tubing or pump syringes during normal operation.

1. Fill a bottle with > 500 ml of 70% isopropanol and another bottle with deionized water for rinsing the tubing.

2. Carefully unscrew the cap of the water reservoir and place the end of the tubing into the isopropanol bottle.

3. Click the [System Prime] button to prime the tubing with isopropanol.

4. Upon completion, remove the cap from the isopropanol bottle and submerge the cap tubing in the bottle with deionized water to rinse off the residual isopropanol.

5. Place the clean tubing back into the water reservoir bottle and screw the cap back on.

6. Run the [System Prime] process two more times (twice) while connected to the water reservoir to flush out the isopropanol and replace with water.
E. Run the Tip Clean Procedure

The cleaning process takes ~2 min.

1. Click the Advanced tab in the ICELL8 cx Software.

2. Observe the “Wash” well of the trough.

![Figure 20. Location of the “Wash” well of the trough.](image)

3. If the well is dry, click the [Wash Prime] button to fill with wash solution.

![Figure 21. The [Wash Prime] button.](image)

4. Click the [Tip Clean] button to initiate the sequence to wash the inside and outside of the tips.

![Figure 22. The [Tip Clean] button under the Advanced tab.](image)

5. After this step is complete, the system is ready for use.
VII. Protocol: Prepare the sample source plates

A source plate is a 384-well plate containing either the samples (i.e., a sample source plate) or the reagents (i.e., a reagent source plate) that are to be transferred using the ICELL8 cx.

**IMPORTANT:** Only the plates included in the ICELL8 384-Well Source Plate and Seal kit (Cat. No. 640192) are validated for use on the ICELL8 cx System.

**IMPORTANT:** Avoid introducing dust and debris to solutions that will be dispensed with the ICELL8 cx. They can cause the tips to clog.

Observe the following precautions when assembling sample and reagent source plates:
- Consider assembling source plates in a dead air box to reduce environmental dust
- Wipe down the lab bench every day and wear gloves and a clean lab coat
- Use plates, tips, and tubes from new or carefully covered containers
- Work quickly and cover plates/tubes to minimize exposure to dust in the air

1. Pipette the cell sample and experimental controls into a 384-well sample source plate. Refer to the plate map shown below (Figure 23).

![Figure 23. Source plate map for cell sample dispense.](image)

2. After filling the wells, seal the plate with a 384-well source plate seal. Do not centrifuge.

**NOTE:** Use source plates immediately, or store on ice.

VIII. Protocol: Dispense samples into the chip

A. Select the ICELL8 cx application

The 3’ DE/TCR protocol will be used to illustrate the basic workflow. Please refer to the application-specific user manuals at takarabio.com for more details on these protocols.

![Figure 24. The 3’ DE / TCR application tab in the cx instrument software UI.](image)
B. Place the chip in the ICELL8 cx unit

1. Visually inspect the chip nest and clean it if there is any debris.

2. Remove the seal on the chip and place the chip on the chip nest. Make sure the chip fits tightly and lays flat (Figure 25).

![Figure 25. Procedure to place the chip in the chip nest.](image)

When loading the chip, it is recommended to wear tight-fitting gloves so as not to interfere with chip placement, and to only touch the edges of the chip, minimizing contact with the wells (top face of chip). To load the chip, first correctly position the chip by setting it down on top of the chip nest with the chamfered corner oriented to the bottom right and the sides of the top right corner of the chip contacting the inner ends of the prongs (Panel A). Then, gently slide the chip toward the top right, into the clip; the arms of the clip will spread out slightly. Once the left and lower sides of the chip clear the alignment posts (Panel B), push the chip down into the chip nest and back towards the alignment posts. To ensure the chip is fully against the alignment posts, push down on all corners of the chip and pull the chip towards the alignment posts (Panel C). Visually check to make sure the bottom face of the chip is flat against the black anodized aluminum surface (“chuck”) and the top face of the chip is flush with the surface of the chip nest. It is important to check that the chip is fully seated before dispensation to avoid interference with the dispenser tips.
C. Place the sample source plate in the ICELL8 cx unit

1. Place the sample source plate on the Plate Nest with the A1 position in the top, right corner (Figure 26). Press down on plate to ensure it is sitting flat against the platform.

![Image of Plate Nest with A1 Position](image)

Figure 26. Empty plate nest for the sample source plate.

2. Close the front access panels of the ICELL8 cx module.; both doors should remain closed during the dispensing process.

![Image of ICELL8 cx with Front Doors](image)

Figure 27. View of the ICELL8 cx with the front doors open (left) and closed (right).

3. Let the system idle for 2 min, until the chamber has reached the relative humidity and dew point setpoints.
D. Dispense the sample cells and experimental controls into the Chip

**IMPORTANT:** Do not open the door of the stage module while the ICELL8 cx is dispensing. If the door is open, the chip can become contaminated. Additionally, when the door is open, evaporation from the nanowells can occur, resulting in changes to concentrations of the reagents in the chip.

1. Click the [Dispense cells] button to initiate the dispense workflow.

![Figure 28. [Dispense cells] under the application-specific protocol tab.](image)

2. A message will pop up reminding the user to make sure the source plate and chip are properly inserted. Click [Done].

![Figure 29. Prompt pop-up to confirm plate and chip loading.](image)

3. The system will check to see if the minimum vacuum level has been reached.

![Figure 30. cx software UI display while checking the minimum vacuum level.](image)
If minimum vacuum level is reached, the status button will show green and automatically continues to step 5.

**Figure 31. Vacuum status confirmation. (Left) Minimum vacuum level has been reached. (Right) Insufficient vacuum level indicator.**

If the vacuum level is insufficient, the vacuum status will show a red dot and an error message will display; Click [Abort] to return to the main menu and run through the following steps:

a. Click [Dispense cells] to restart the sequence. Follow the prompt by pushing the chip down and visually verifying that it is fully seated on the chuck. Then click [Done].

b. If an error message indicates that vacuum levels are still not reached, but the chip is fully seated, click [Yes] to proceed to step 5.

4. The software will remind the user to remove seals (on chip and source place). Click [Done] in the **Remove seal** window to confirm.

**Figure 32. Confirm seal removal pop-up.**

5. The system will perform autofocus and barcode read steps. The live image of chip scanning will display for user reference. The scanning process will take 1–2 minutes.

**Figure 33. Live image of chip scanning.**
NOTE: Occasionally if the chip is not inserted correctly, the software will prompt the user to make sure that the chip is fully seated against the 3 alignment posts and oriented correctly. If this happens, click OK. Open the door to the cx and visually check that the chip is sitting flat (refer above to Figure 25, Panel C). Restart the workflow by clicking the [Dispense cells] button.

![Pop-up message](image)

**Figure 34. Pop-up message if a problem is detected during the chip scan.**

6. If the scan completes but the software is unable to read the chip ID, manually enter the ID into the “Chip ID” field. Click [Done] to proceed to the dispense.

![Input chip ID](image)

**Figure 35. Input chip ID.**

7. When the dispense is complete, the vacuum should turn off automatically. You can then remove the chip for blotting.

![Vacuum status](image)

**Figure 36. Vacuum status after dispense.**

8. Perform the tip clean procedure 3-4 times (see Section VI.E)
E. **Blot and centrifuge the chip**

1. After sample dispense is complete, promptly blot the chip for 2 sec, as described below.
   a. Place the chip, wells facing up, on a clean lab wipe.
   b. Gently place a piece of blotting paper directly on top of the chip. Make sure that the blotting paper covers the entire face of the chip.
   c. Pick up the blotter by the top handle and place the flat face of the blotter against the blotting paper on the chip. The blotter should extend beyond the edges of the chip.
   d. Let the blotter rest on top of the blotting paper for exactly 2 sec without pressing down; the weight of the blotter is sufficient for adequate blotting.
   e. Remove the blotter, then gently remove the blotting paper and dispose of it in a biohazard container.

2. Quickly seal the loaded chip with appropriate film (specified by the protocol).

3. Centrifuge as described below.
   a. Place your chip on the centrifuge tray.
   b. Counterbalance with a second chip.
   c. Centrifuge at 300g for 5 min at 22°C.

F. **Scan chip for single cells and freeze the chip**

1. After centrifugation of the chip with the samples is complete, remove the film on the chip and load the chip in the chip nest.

2. Press [Scan chip] to initiate the cell scan workflow.

![Figure 37. Initiate chip scanning.](image-url)
3. Similar to the cell dispense workflow, the software will:
   a. Prompt the user to verify that the chip is inserted properly.
   b. Check that sufficient vacuum levels have been reached.
   c. Remind the user to remove the seal on the chip.
   d. Perform autofocus for chip barcode.

4. When the autofocus and barcode scanning steps are complete, if the software was unable to read the ID, manually enter the chip ID into the “Chip ID” field.

![Image of workflow with chip ID entry](image)

Figure 38. Enter the chip ID after autofocus and barcode scanning steps.

5. Select the proper analysis setting and barcode .gal or .xml files. Press [OK] to confirm.

![Image of new stack info with analysis setting](image)

Figure 39. Selecting the analysis setting and barcode files.
6. Select the path and the name of the folder where data will be saved. Press [OK] to confirm.

**NOTE:** Make sure that the “Base folder” path is set to a location other than the C: \ drive. The scan folders are large and will quickly fill up the C: \ drive if set there.

7. Chip scanning will begin. (The scanning channels are pre-defined in a configuration setting.) Contact TBUSA technical support (technical_support@takarabio.com) for more details.

8. When chip scanning is complete, the system will ask if you want to save the result file. Press [Save] to proceed.

9. Name the results file. This folder will contain the filter file which will be used in the next step to define which wells contain single cells and should be dispensed to.

**Figure 40.** Select path and file name for saving data.

**Figure 41.** Naming and saving results file.
10. The vacuum should turn off automatically when scanning is complete. You can remove the chip at this time for the freezing step.

11. Close only the ICELL8 cx CellSelect Software GUI and image viewer, **NOT** the main ICELL8 cx System software.

**G. Dispense reagents into the chip**

The next steps are representative of the dispense of most downstream reagents. The example used is the RT dispense for the 3’ DE/TCR protocol.

1. Check that the GUI is on the correct tab.

![Figure 42. Cell / RT dispense and scanning.](image)

2. Before the dispense step, remove the chip and plate seals, place the chip securely flat on the chip nest and load the 384-well plate on the plate nest. Refer to the plate map shown below (Figure 43).

![Figure 43. RT-PCR mix Source Plate map.](image)

3. Close the front door of ICELL8 cx unit.

4. Click the [Dispense RT - PCR mix] button to initiate RT dispense.

![Figure 44. Initiate RT dispense.](image)
5. Select the filter file that was generated from the cell scan step (previous step, Section VIII.F). Press [Open], then [Done] on the confirmation dialogue window.

Figure 45. Selecting (top) and loading (bottom image) the filter file.

Similar to the Cell dispense workflow, the software will:

a. Prompt the user to verify that the chip is inserted properly.
b. Check that sufficient vacuum levels have been reached.
c. Remind the user to remove the seal on the chip.
d. Perform autofocus for the chip barcode.
6. When the autofocus and barcode scanning steps are complete, if the software was unable to read the ID, manually enter the chip ID into the “Chip ID” field. **Figure 46. Enter chip ID.**

**NOTE:** If the chip ID entered does not match the filter file name, the software will give you the option to proceed or abort. Click [Yes] or [No], as appropriate. **Figure 47. Confirm that chip ID matches filter file name.**

7. After confirming the chip ID confirmation, the RT dispense will begin.

8. When the dispense step is complete, the vacuum should turn off automatically; you can then release the chip.

9. Carefully remove the chip from the chip nest.

10. Blot and seal the chip.

11. Perform the tip clean procedure 3-4 times (Section VI.E).

H. **Run your PCR on a thermal cycler**

If you are using the ICELL8 cx Single-Cell System (Cat. No. 640188), use the thermal cycler (Cat. No. 640002) included with the system. See the user manual shipped with the thermal cycler for complete instructions on thermal cycling the filled chip.

If you are using the ICELL8 cx Single-Cell System JPN (Cat. No. 640189), use a thermal cycler of your choice. See the manufacturer’s user manual for further instructions on thermal cycling the filled chip.
I. Clean the ICELL8 cx System

1. After each dispense, remove the 384-well plate from the plate nest and properly dispose of it.

2. Visually inspect the dispensing platform for any debris. Moisten a cloth with 70% isopropyl alcohol then wipe down the dispensing platform (chip nest, source plate nest, trough, and the areas on the base platform immediately surrounding them). Do not wipe the plastic side panels.

   **WARNING:** Take care not to bump the tip nozzles while wiping down the dispensing platform. This could put the tips out of alignment and result in poor dispenses in the future. If this occurs, please contact Technical Support (technical_support@takarabio.com).

   **WARNING:** Ensure that no liquid drips below the platform, especially onto the temperature and humidity sensor, which is located just to the left of the chip nest.

3. After the final dispense of the day, perform the tip clean procedure (Section VI.F).

   **NOTE:** It is recommended to leave the instrument and software ON when not in use.

IX. Maintenance

**CAUTION:** There are no user-serviceable parts inside the instrument. Service of internal parts should be performed by a qualified TBUSA service technician.

A. Daily Maintenance

Daily Maintenance procedures help ensure optimal instrument operation and prevent problems. They are described in “Protocol: Preparing the ICELL8 cx System” in Section VI.
ICELL8® cx Single-Cell System User Manual

B. System Shutdown Procedure

It is recommended to leave the instrument and software ON. If the ICELL8 cx needs to be shut down for long periods of time, the following procedure can be performed by qualified personnel.

**WARNING:** Moving the instrument may damage its enclosure if not done properly. Please contact TBUSA technical support for more details.

1. Follow the ICELL8 cx System cleaning procedure described above in Section VIII.I.
2. Empty the contents of the water and humidifier reservoirs.
3. Press the [System Prime] button to empty the system tubing of water.
4. Dispose of the contents of the wash bottle.
5. On the *Advanced* tab, press [Wash Prime] until there is no new fluid coming up from the wash well.
6. Exit the ICELL8 cx Software by clicking the [Close] button at the top right of the window.
7. Turn off the ICELL8 cx unit by toggling the power switch on back of the system.

![Figure 49. Location of the system power switch.](image)

8. Check that all nanowell chips and source plates have been removed from the stage module.
9. Properly dispose of contents of waste container, rinse, and allow it to dry.
10. Remove the back cover, connect a tube to the drain valve on the humidifier tank, and drain the humidifier.
11. Power down the computer that controls the ICELL8 cx instrument.

C. Annual Preventative Maintenance

Have the ICELL8 cx examined and calibrated every year by a TBUSA service engineer.
Appendix: Troubleshooting

If the ICELL8 cx or its software does not respond as desired or a warning is displayed, please attempt to rectify the problem using the tables below. If you cannot solve the problem, contact TBUSA technical support.

A. Diagnosis

Table V. Problem: Poor dispensing, excessive fluid on top of chip.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trapped air in the system fluidics</td>
<td>Inspect the tubing between the tip and the 2 x 4 manifold (where not covered). Look for air or bubbles within the tube. If air is found within the tube, run the [Daily Warmup] 2-3 times. If the air bubble fails to be flushed out, perform a System Prime with isopropanol. Refer to Section VI.D for priming procedure.</td>
</tr>
<tr>
<td>Plugged tip</td>
<td>Run the tip clean procedure times (Section VI.E). If the tip is plugged with a soluble material, aspirating and dispensing reagent capable of dissolving the material may clear the blockage. If this measure fails, contact TBUSA technical support.</td>
</tr>
<tr>
<td>Leaking inlet valve</td>
<td>Let the system sit idle for 5 min and inspect the dispensing tip nozzles. If there are water droplets accumulating at any of the tips, it is possible that the solenoid is leaking. Contact TBUSA technical support.</td>
</tr>
<tr>
<td>Incompatible buffers and samples</td>
<td>Extremes of fluidic properties, such as viscosity, may result in poor performance. Contact TBUSA technical support.</td>
</tr>
<tr>
<td>Crimped fluidic tubing</td>
<td>Inspect the tubing for crimps or bends. Straighten out crimped/bent sections if feasible. Contact TBUSA technical support if tubing needs to be replaced.</td>
</tr>
</tbody>
</table>

Table VI. Problem: Dispensing head does not home.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No communication with the instrument</td>
<td>Check the power cables to the cx instrument and the network switch are firmly plugged in on the ICELL8 cx unit. Close the ICELL8 cx software, power cycle the instrument, and restart the software.</td>
</tr>
</tbody>
</table>

Table VII. Problem: Low or partial dispenses.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsolenoid valve failure</td>
<td>Contact TBUSA technical support.</td>
</tr>
<tr>
<td>Crimped tubing</td>
<td>See Table VI above.</td>
</tr>
</tbody>
</table>
Table VIII. Problem: Apparent low sample concentration.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe thumbscrew is loose</td>
<td>Tighten the syringe thumbscrew until finger tight. The thumbscrew is located at the bottom of the syringe in the syringe pump unit.</td>
</tr>
<tr>
<td>Tip is plugged</td>
<td>See Table VI above.</td>
</tr>
<tr>
<td>Air bubble in the syringe path</td>
<td>Prime the syringe path by performing the Daily Warmup.</td>
</tr>
<tr>
<td>Microsolenoid valve is leaky</td>
<td>See “Leaking inlet valve” (Table VI) above.</td>
</tr>
<tr>
<td>Syringe valve is blocked or leaky</td>
<td>See “Leaking inlet valve” (Table VI) above.</td>
</tr>
<tr>
<td>Low liquid level in the Water Container</td>
<td>Check the level of the system liquid in the Water Container. Make sure that the end of the tubing is submerged in the water. Add deionized, degassed water as needed (See “Powering on the System” in Section VI.A).</td>
</tr>
</tbody>
</table>

Table IX. Problem: System stalls because the syringe does not move.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No power</td>
<td>Check the power cables to the cx instrument and the network switch are firmly plugged in on the ICELL8 cx unit. (See Figure 1 in Section I.D)</td>
</tr>
<tr>
<td>Syringe is not initialized</td>
<td>Close the ICELL8 cx software, power cycle the instrument, and restart the software.</td>
</tr>
<tr>
<td>Obstruction</td>
<td>Verify that there is no object obstructing the movement of the syringe.</td>
</tr>
</tbody>
</table>

B. Technical Support

If you require technical support, please contact your authorized TBUSA service technician, or contact us directly at: technical_support@takarabio.com.

Contact Us

<table>
<thead>
<tr>
<th>Customer Service/Ordering</th>
<th>Technical Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>tel: 800.662.2566 (toll-free)</td>
<td>tel: 800.662.2566 (toll-free)</td>
</tr>
<tr>
<td>fax: 800.424.1350 (toll-free)</td>
<td>fax: 800.424.1350 (toll-free)</td>
</tr>
<tr>
<td>web: takarabio.com</td>
<td>web: takarabio.com</td>
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<td>e-mail: <a href="mailto:ordersUS@takarabio.com">ordersUS@takarabio.com</a></td>
<td>e-mail: <a href="mailto:technical_support@takarabio.com">technical_support@takarabio.com</a></td>
</tr>
</tbody>
</table>

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