Takara Bio USA, Inc.

ICELL8® MultiSample NanoDispenser User Manual

Cat. No(s). 640000, 640147

(080519)

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

Table of Contents

Table of Figures

Table of Tables

I. Introduction

Congratulations on the purchase of your Takara Bio ICELL8 Single-Cell System. This manual covers the use of one component of the system, the MultiSample NanoDispenser. There are two types of MultiSample NanoDispensers that may be part of the ICELL8 system, an older "MSND" and a newer "MSND+". The instructions covered in the manual are identical for both, so in this document, MSND refers to either version.

The MSND is designed to load samples into ICELL8 chips.

A. Safety

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

1. Operating Conditions

The instrument is safe to operate with the covers in place. The covers protect the user from live 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 MSND only inside an appropriate building. Do not operate the MSND outside or in wet environments.

2. Instrument Use

- Use of the MSND may cause exposure to toxic or biohazardous chemicals thereby presenting a hazard. Wear appropriate personal protective equipment (PPE), which should at a minimum include gloves, eye protection, and lab coat at all times in the laboratory.
- Class I Equipment: This equipment must be earthed. The power plug must be connected to a properly wired earthed ground socket outlet. An improperly wired socket outlet could place hazardous voltages on accessible metal parts.
- Do not position the equipment so that it is difficult to operate the power switch or remove the power cord.
- Use only the power cord provided by the manufacturer. Do not replace the power cord with an inadequately rated cord.

3. Moving and Lifting the System

If you need to move the system after it has been installed, use proper lifting techniques and appropriate moving equipment. More than one person may be required, particularly when moving the Stage Module.

4. Warning Labels on the Instrument

Please note the warning label on the instrument.

WARNING: The system contains moving parts. Keep hands away from the system while the instrument is in use.

B. Certification and Standards Information

The MSND fulfills the following requirements: EN 61010-1:1993 + A2:1995/IEC 61010-1:1990 + A1:1992 + A2:1995

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 2500 V for a 230 V supply and 1500 V for a 120 V supply.
- Pollution Degree 2 according to IEC 60664- 1.
  Pollution Degree 2 assumes that normally only nonconductive pollution, such as dust, occurs with the exception of occasional conductivity caused by condensation are present in the operating environment.

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

II. List of Components

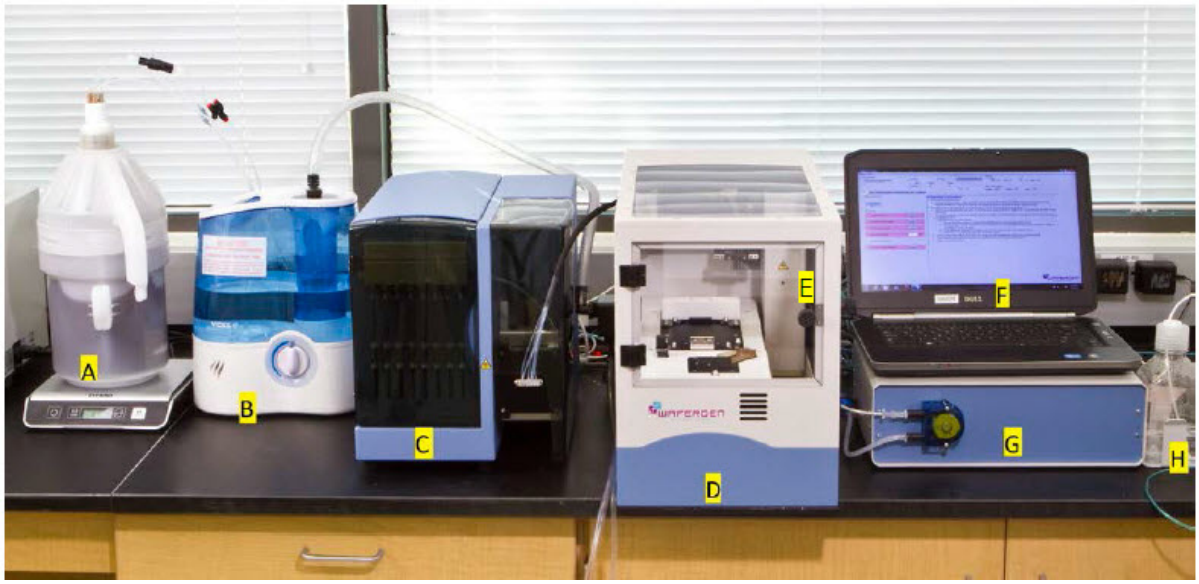

Figure 1. Components included in the MultiSample NanoDispenser (MSND)

The MSND includes the following components (from left to right):

A. Pressure reservoir and Electronic Scale	B. Humidifier	C. MSND fluidic module
D. MSND Stage Module	E. Environmental Chamber	F. Laptop Computer
G. Peristaltic pump control box	H. Wash bottle	

Other items not shown include:

- Barcode reader
- Fluidic Harness and Power Cord
- Waste Container
- ICELL8 system software
- User Manual (this document)
- Digital Pressure Regulator (DPR)
- Tool Set
- Blotter
- Balance Plate

A. Pressure reservoir and Electronic Scale

Figure 2. Pressure reservoir on the electronic scale

The pressure reservoir contains helium-pressurized, deionized and degassed deionized water (or equivalent) that occupies all fluid paths in the fluidic module. The liquid is used to draw and push air gaps and reagents through the harness and tip. The pressure reservoir sits on an electronic scale that monitors water level so that users can make sure that there is enough water prior to starting a chip-dispense operation.

B. Humidifier

The Humidifier maintains the relative humidity at ≥30% in the Environmental Chamber to minimize reagent evaporation during the dispensing process.

C. Fluidic module

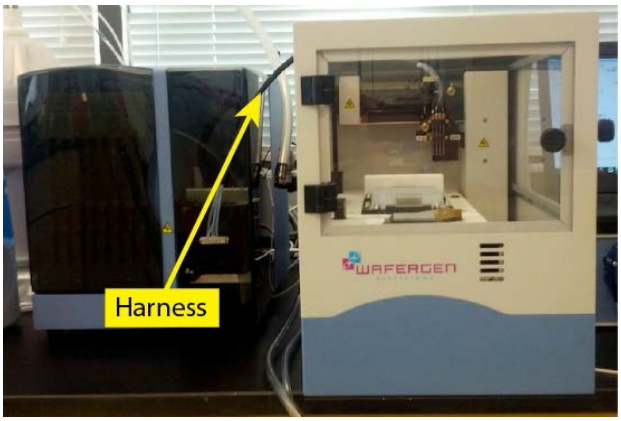

Figure 3. Fluidic and stage modules.

The fluidic module is a hydro-pneumatic system that controls the aspiration and dispensing of samples and reagents in the Stage Module. A tubular harness connects the two modules. Helium pressure and solenoid valves are used to closely control liquid dispensing. The fluidic module also regulates helium to the pressure reservoir through a digital pressure regulator

D. MSND Stage Module

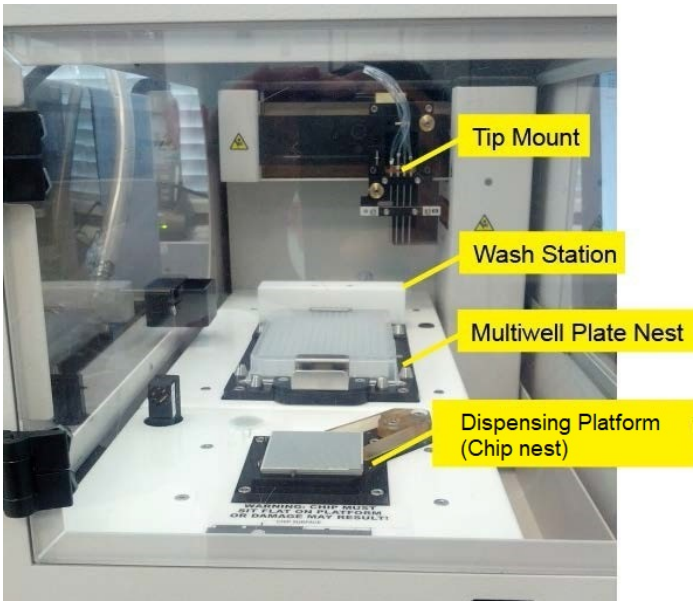

Figure 4. The MSND stage module

The Stage Module houses the head, tips, dispensing platform, multi-well plate nest, wash station, and tip mount used for aspirating reagents and dispensing them into an ICELL8 chip. A single interface cable facilitates mechanical control between the two modules. An environmental chamber surrounds the Stage Module to maintain optimal humidity levels during reagent dispensing.

E. Peristaltic pump control box and environmental controller

Figure 5. Front view of the peristaltic pump control box and environmental controller

The peristaltic pump control box includes one peristaltic pump, which pumps wash solution into the MSND during the tip washing cycles. There are two proportional-integral (PI) controllers inside the pump control box that monitor the enclosure temperature, relative humidity (RH) and chip temperature. They adjust the RH and chip temperature to minimize evaporation during sample dispensing.

The rear of the pump control box has the connections to other components of the system, as shown below.

Figure 6. Rear view of the peristaltic pump control box and environmental controller with connection ports numbered

The connections are:

1. IO: connection to the peristaltic pump
2. SENSORS: connection to the temperature and humidity sensors in the Stage Module
3. MSND 1, MSND 2: connection to the fluidic module
4. TEC: connection to the Stage Module
5. SCALE: connection to the electronic scale
6. VACUUM: connection to the helium supply
7. COMPUTER: USB connection to computer
8. HUMIDFIER: power connection to humidifier

F. Wash bottle

The wash bottle contains molecular biology grade water that is used during the tip-cleaning steps of the dispensing protocol to prevent cross-contamination.

G. MSND Specifications and Lab Requirements

Category	Specification
Dispense volume	35–50 nL or 100 nL per nanowell
Software	ICELL8 system software
Laptop Computer	Windows 7, 2 GB memory, 120 GB storage, 1 GB network adapter, and USB ports for memory sticks.
Power Requirements **(For different power supply types)**	• 120 VAC / 60 Hz mains: one 120V, 15 or 20A circuit. Three NEMA 5–15 receptacles are required for the fluidic module, Pump Box, and Laptop Computer. (The humidifier plugs into the pump box, and thus a separate receptacle is not required.) • 220–240 VAC / 50 Hz mains: one 10A circuit to power a 230:115 step–down transformer and NEMA 5-15 power strip (transformer and power strip are supplied with the system). Transformer adapters will be supplied for Continental Europe (Schuko type), UK, and China installations. • 100 VAC / 50–60 Hz mains: one 15A circuit to power a 100:120 step-up transformer and NEMA 5-15 power strip (transformer and power strip are supplied with the system). • Transformer suitable for Japanese power receptacles.
Fuses	Dispenser: 5 x 20 mm, T5H 1.6 watts / 6.3 A max 250V
Environmental conditions	• Ambient Temperature: 15–30°C • Relative Humidity, non-condensing: 30–70% • Altitude: <2000 m from sea level • Pollution degree: 2 or less
Dimensions	• Laptop computer: 13” W x 2” H x 10” D (34 x 5 x 25 cm) • Fluidic module: 11” W x 13” H x 18” D (28 x 33 x 45 cm) • Stage Module: 11” W x 16” H x 24” D (27 x 40 x 60 cm) • Peristaltic pump control box: 10” W x 15” H x 21” D (26 x 38 x 51 cm)
Bench space	• Bench space including clearance (capable of supporting approximately 110 pounds (50 kg), including CPU) • Dispenser, pump box, CPU, pressure reservoir, transformer (if required): 70” W x 30” D x 24” H (180 x 75 x 60 cm)
Floor space	• Humidifier: 16” W x 32” H x 26” D (41 x 81 x 66 cm) • Helium Source: 10” diameter cylinder (or equivalent) x ~60” H (25 x 152 cm) • Waste Container: 8 3/4” W x14 1/8” H x 6” D (22 x 36 x 15 cm)
Weight	143 pounds (65 kg)
Reproducibility	Takara Bio Standard Positive Control DNA Test: Ct SD <0.25

H. Setup and Installation

Your Takara Bio Service Engineer will unpack and install your ICELL8 MSND and explain the basic operation of the system. They will use material from the Nanodispenser Installation Kit (Cat. No. 640012) to qualify the instrument after installation and will leave reusable and/or remaining materials at your site. The following table lists the Nanodispenser Installation Kit components.

Component

- Nanodispenser Alignment Chip (1)
- Nanodispenser Alignment Chip Film (pack of 10)
- Imitation Mastermix with UV and Rox Dyes
- 384-Well Source Plate (2)
- 384-Well Source Plate Seal (2)
- Blotting Paper (pack of 10)

The computer that runs the ICELL8 MSND is equipped for Wi-Fi access, but it is disabled. If you choose to activate Wi-Fi, we recommend that you seek support from your institution's IT personnel to avoid interfering with instrument operation.

NOTE: To avoid contaminating your PCR reactions, do not install the MSND in an area that could contain high copy DNA or amplicon from previous PCR reactions.

I. Required Equipment and Supplies from Your Lab or Other Suppliers

1. Helium

- 99.9% purity or greater
- Capacity: approximately 223 standard cubic feet (reported at 15.6 °C and 1 atmosphere [1.01325 Bar]). This capacity is sufficient for six months or more of typical usage.
- Pressure: 2,265 psi (150 Bar), with regulation from 0 to 200 psi (0–15 Bar). Typical usage pressure 30 psi (2 Bar). Use a regulator such as the Concoa regulator (P/N 3124311-01-580).
- Fittings to accommodate 3.2 mm outer diameter flexible urethane tubing fittings (push-to-connect fittings). Acceptable thread forms are 1/8" NPT (female) or M5 straight thread (female).

2. Wash solution

- Molecular biology grade water

3. Reagents for MSND Reservoirs

- Pressure reservoir: Fill with deionized filtered water
- Wash bottle: Fill with wash solution

4. Other Reagents and Materials

- Prepared sample reagent mixtures.
- Instructions for preparing samples and reagents for dispensing with the ICELL8 MSND are provided with application specific user manuals.
- 70% isopropanol

5. Equipment

- Ice bucket and/or cold rack
- Calibrated pipettor and nuclease-free, aerosol-resistant tips. (8-channel and repeating pipettors are very useful in this procedure.)
- Vortex mixer
- Centrifuge with a rotor capable of spinning microwell plates at 3220 RCF (x *g*)

J. Required Materials from Takara Bio

To order, visit our website at takarabio.com or contact customer service.

Table I. Required materials for all applications

Cat #	Product name	# of plates	Description
640018	MSND 384-Well Source Plates and Seals	20	These specific 384-well plates are the required container for solutions that will be dispensed using the MSND. Includes plate seal.
640037		120	
640192	ICELL8 384-Well Source Plate and Seal	5	

K. SmartChip Technology

SmartChip technology distinguishes the Takara Bio platform from other systems. Each SmartChip (also referred to as "ICELL8 chip" or "chip") has a 72 x 72 array of nanowells and can accommodate up to 5,184 100-nL real-time reactions in a single run.

The MSND transfers samples, controls, and reagents from the 384-Well Plate to the ICELL8 chip. After dispense, the chip can be processed for cell analysis or library.

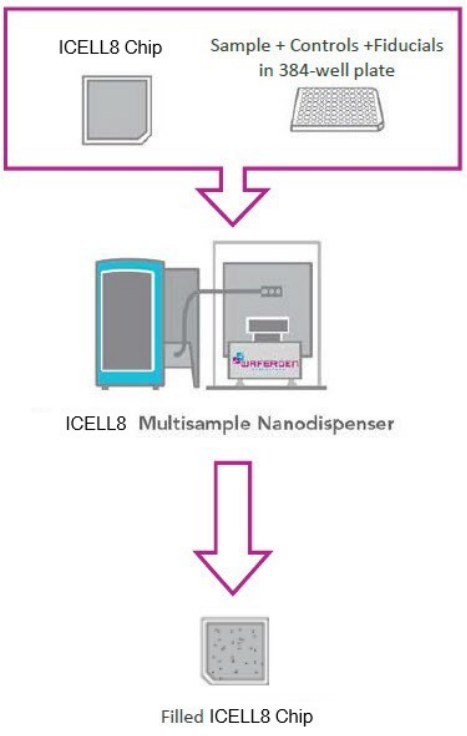

Figure 7. Simplified workflow

III. Procedure

The following table is a quick reference guide for dispensing samples into the ICELL8 chip. Print this guide for easy reference in the laboratory. Each step is described in detail in the sections following the quick reference guide.

Table II. ICELL8 MSND quick reference guide

1. **Prepare the ICELL8 MSND.**
   a. Power on the System
   b. Check and fill system containers
   c. Run Daily Warmup
   d. Run Tip Clean procedure
2. **Prepare source plate for an ICELL8 chip**
   a. Pipette cell sample into a 384-well sample source plate.
   b. Seal plate with imaging film. Do not centrifuge.
3. **Dispense sample into the ICELL8 chip.**
   a. Enter chip ID into software
   b. Place the chip into the MSND.
   c. Place the Sample Source plate into the MSND.
   d. Dispense sample cells, fiducial, and controls into chip.
   e. Blot and seal chip.
   f. Image and then freeze the chip.
4. **Prepare source plate for RT mix.**
5. **Dispense RT mix into the ICELL8 chip.**
6. **Run your PCR reactions on the ICELL8 Thermal Cycler**
7. **Clean the MSND**

A. Prepare the MSND

1. Power on the System

IMPORTANT! Make sure that the pump box is connected to the proper USB port on the computer (marked) with a USB cable.

1. Power on the fluidic module and the Pump Box using the switches on the back of the components.
2. Power on the computer and start the ICELL8 system software on the computer.

2. Check System Containers

It may take ~5 min for the MSND dew point sensors to stabilize and the system to become available.

1. Check helium tank pressure. The regulator should have a supply input (on the side closer to helium tank) of >500 psi (3.5 MPa), and an output (side closer to the MSND) of ~30–40 psi (0.24 MPa). If the helium tank pressure drops below 500 psi, replace the tank.
2. Check the amount of water in the pressure reservoir.
   a. Open the top of the protective cover on the pressure reservoir. Be careful not to misplace the O-ring or damage the tubes coming from the lid.
   b. Tilt the bottle sideways to check the amount of water in the reservoir. It should be at least half full of deionized filtered water at the beginning of a run.

   If needed, add water to the reservoir (see "Refill the pressure reservoir", Section III.A.3, below).

Figure 8. The pressure reservoir and its protective cover

3. Check the waste container. If full, dispose of waste appropriately and replace with an empty waste container.
4. Check the wash bottle. If there is less than ~1 inch (2.5 cm) of liquid in the wash bottle, add Molecular Biology grade water to the 500 mL mark (see Figure 9).

Figure 9. The wash bottle, illustrating a low level and the fill line

5. Check the humidifier reservoir. If the level of the water in the humidifier reservoir is less than 2 inches (5 cm) from the bottom of the reservoir, add water to the reservoir (see "Add Water to the Humidifier Reservoir", Section III.A).

Figure 10. The humidifier reservoir and the level at which the reservoir should be refilled

6. Check the system humidity.
   a. Close all the doors to the Environmental Chamber.
   b. Rotate the humidifier control switch all the way to the right (clockwise), to the maximum setting. The system will not dispense unless the humidity is ≥ 60% RH.

3. Refill the pressure reservoir

1. Put on clean gloves.
2. Vent the helium by closing the stopcock on the helium-in line and opening the vent stopcock.

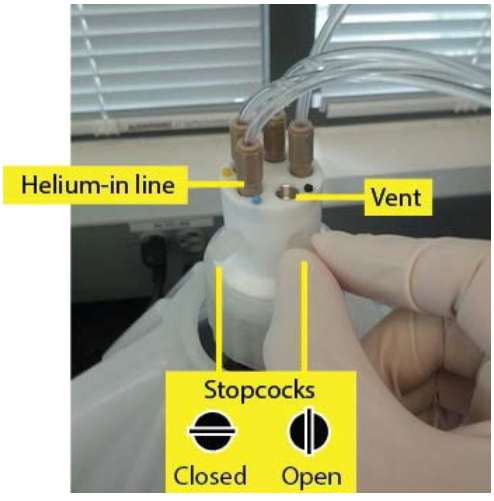

Figure 11. Helium tubing connection scheme and stopcock positions

3. Open the top of the protective cover. There is no need to remove the entire tubing harness from the reservoir.

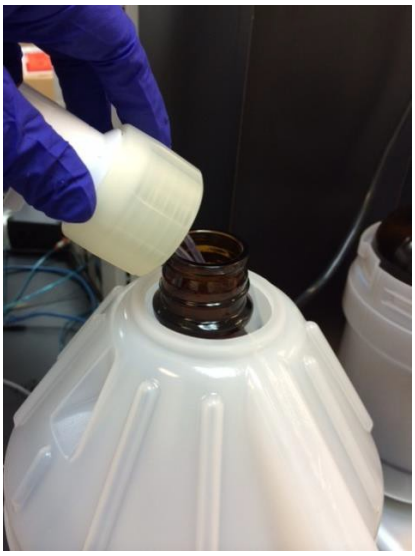

Figure 12. Opening the protective cover of the pressure reservoir without removing the tubing harness

4. Using a graduated cylinder, fill the bottle with deionized filtered water to the top of the lower part of the protective cover.

Figure 13. Filling the pressure reservoir using a graduated cylinder.

Figure 14. Level at which the bottled should be filled

5. Reattach the cap, replacing the tubes inside the reservoir.
6. Reattach the top of the protective cover.
7. Let the reservoir liquid de-gas for 30 minutes. You should see helium bubbling through the water during this period.
8. Close the system by opening the stopcock on the helium-in line and closing the vent stopcock.

4. Add Water to the Humidifier Reservoir

1. Unplug the hose adapter from the top of the humidifier.

Figure 15. Hose adapter unplugged from the top of the humidifier

2. Fill the reservoir with deionized filtered water.

Figure 16. Filling the humidifier reservoir. The photo on the right shows to the recommended capacity.

3. Close the cap securely and place the reservoir back onto the humidifier base unit.

Figure 17. Close the humidifier reservoir cap

4. Replace the hose adapter.

5. Run the Daily Warmup

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

The Daily Warmup takes approximately 8 minutes.

1. Click the `Startup` tab in the ICELL8 system software.

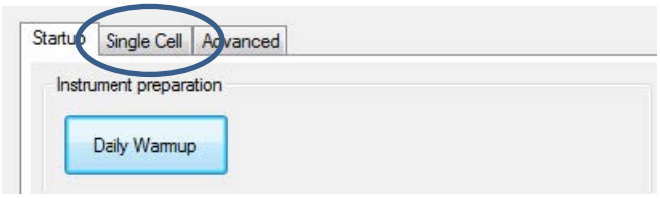

Figure 18. ICELL8 system software, where to locate the [Daily Warmup] button

2. Click the [Daily Warmup] button in the Instrument preparation section. The MSND does the following:
    - Displays a dialog in the software indicating that the system is being brought up to pressure.
    - Sends the head to the Purge position on the MSND platform,
    - Primes the syringe path once.
    - Purges the syringe valves to remove any air that may be trapped in the syringe valves.
3. During the Daily Warmup, monitor the syringes in the fluidic module for trapped bubbles. Small bubbles are acceptable, but larger bubbles are not. In the photos below, the bubble on the left is an acceptable size, while the bubbles on the right are too large.

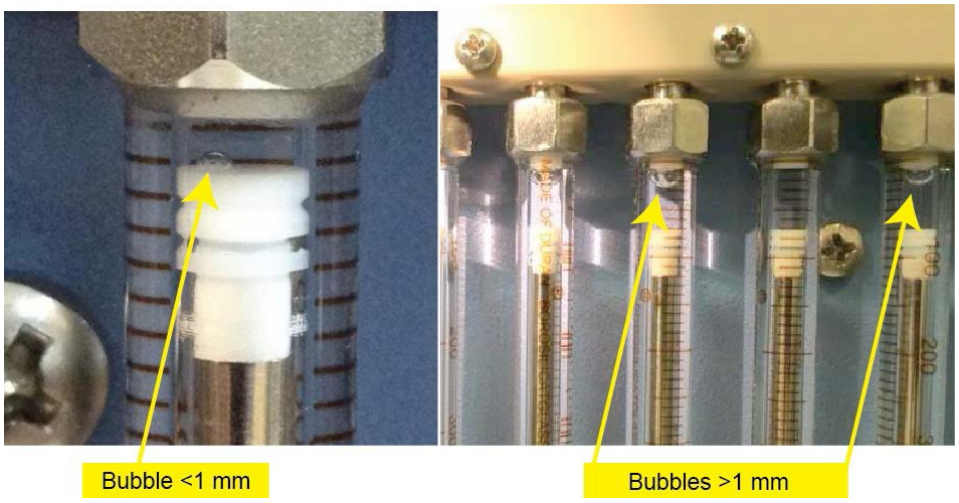

Figure 19. Fluidic module: acceptably sized small bubbles (left), bubbles that are too large (right)

If bubbles larger than 1 mm are observed:

a. Allow the Daily Warmup procedure to finish.
b. Repeat the first portion of the Daily Warmup procedure.

4. Click [System Prime] button.

5. After priming is complete, click [Daily Warmup]. If bubbles persist, repeat Step 2 above until all bubbles larger than 1 mm are purged from the syringe bank.

NOTE: If large bubbles are still present after three Daily Warmup cycles, Takara Bio recommends an isopropyl alcohol wash (see Appendix: Alcohol Wash Procedure).

6. Run the Tip Clean Procedure

The cleaning process takes approximately 2 minutes.

1. Click the `Advanced` tab in the ICELL8 system software.
2. Click the [Tip Clean] button in the Manual Control section.

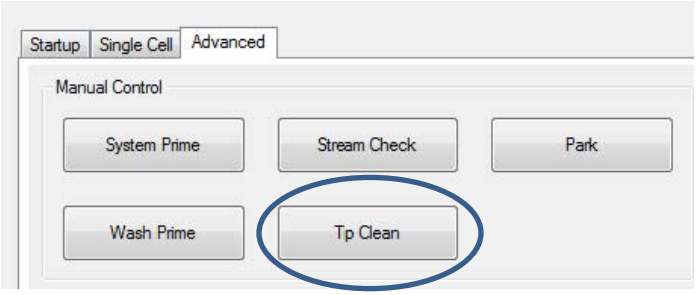

Figure 20. ICELL8 system software *Advanced* tab menu

The system cleans the tips in the wash station for 3 rinse cycles.

B. Prepare the Source plates

A "source plate" is a 384-well plate containing either the samples (a sample source plate) or the PCR assays (an assay source plate) that are to be transferred using the MSND.

IMPORTANT! Only MSND 384-Well Source Plates (Cat. Nos. 640018, 640037, and 640192) are validated for use on the MSND.

IMPORTANT! Avoid introducing dust and debris to solutions that will be dispensed with the MSND; they can cause the tips to clog.

Observe the following precautions when assembling sample and assay 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 cell sample (for genotyping), controls, and fiducials into a 384-well sample source plate. Refer to the plate map shown on the Single Cell tab or shown below.

NOTE: Use source plates immediately or store on ice.

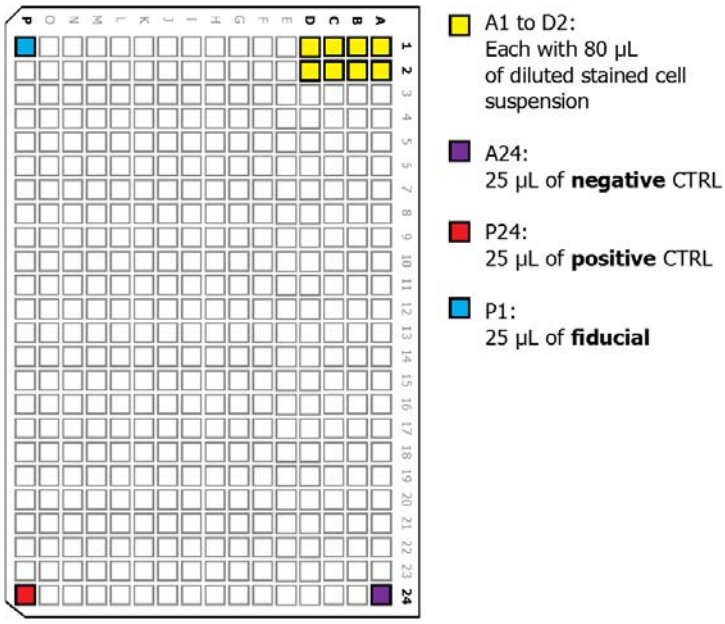

Figure 21. 384-Well Plate map locations for cell sample, negative and positive controls, and fiducial solution.

2. After filling, seal plate(s) with ThermalSeal TSTM 384-well plate seal. Do not centrifuge.

C. Dispense Sample into the SmartChip

1. Enter chip ID into software

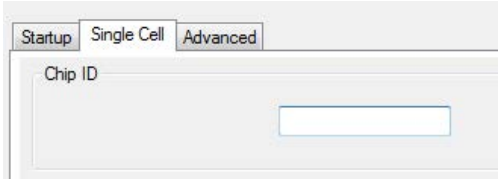

Figure 22. ICELL8 system software: Single Cell tab

1. Click the Single Cell tab.
2. Enter the Chip ID. You can enter the Chip ID by typing in the text field or by using a barcode reader.

Figure 23. Chip ID field in the ICELL8 system software.

To use a barcode reader:

a. Place your cursor in the `Chip ID` text field.
b. Scan the 2-D barcode on the back of the chip.

The Chip ID can be used later to identify the well layout file for this chip.

2. Place the ICELL8 chip onto the MSND

1. Remove the protective film from your ICELL8 chip.
2. Visually inspect the dispensing platform and clean it if there is any debris.
3. Place the chip on the dispensing platform in the Stage Module.
   a. Stretch the arms of the clip apart and angle the chip onto the dispensing platform with the beveled corner in the lower right corner and the edges of the chip pressed against the three alignment pins.
   b. Carefully release the clip that holds the chip in position.

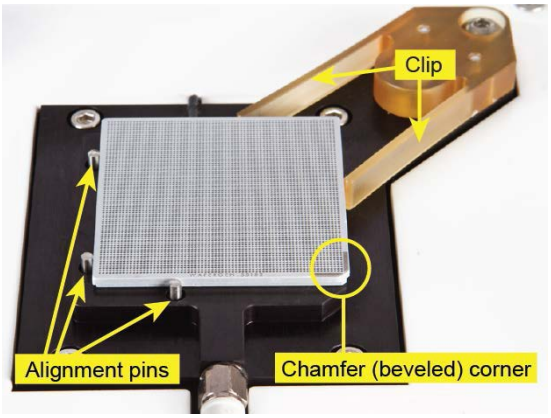

Figure 24. Alignment of the ICELL8 chip on the dispensing platform. Note the positioning of the chamfered corner.

3. Place the Sample Source plate into the MSND

1. Remove the adhesive film.
2. Place the sample source plate on the plate nest with the A1 position in the top, back right corner.

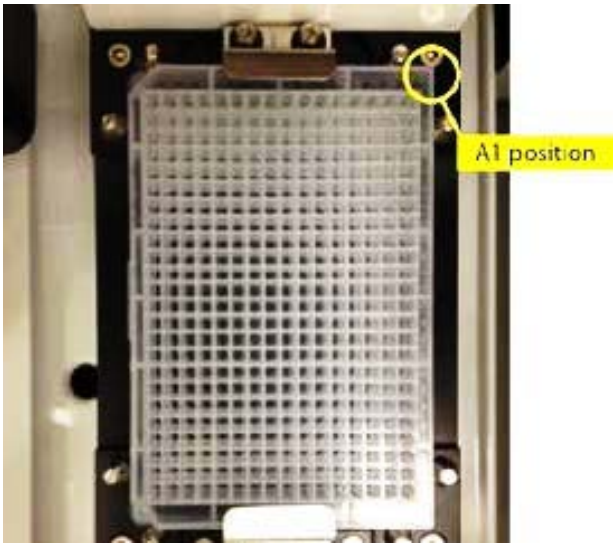

Figure 25. Placement of the source plate on the plate nest.

3. Close the environmental chamber doors.

4. Dispense sample cells, fiducial, and controls into the ICELL8 chip

IMPORTANT! Do not open the door of the Stage Module while the MSND 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 ICELL8 chip.

IMPORTANT! Do not touch the barcode reader while the MSND is dispensing. Operating the reader while the system is dispensing could interfere with the instrument run.

1. Click the *`Single Cell`* tab in the software (if it is not already displayed).
2. Click the [Dispense Cells] button. The software confirms the dispense volume that will be used. Click [OK] to dispense. The MSND will start dispensing 50 nl of cell suspension, control, and fiducial to the appropriate nanowells.

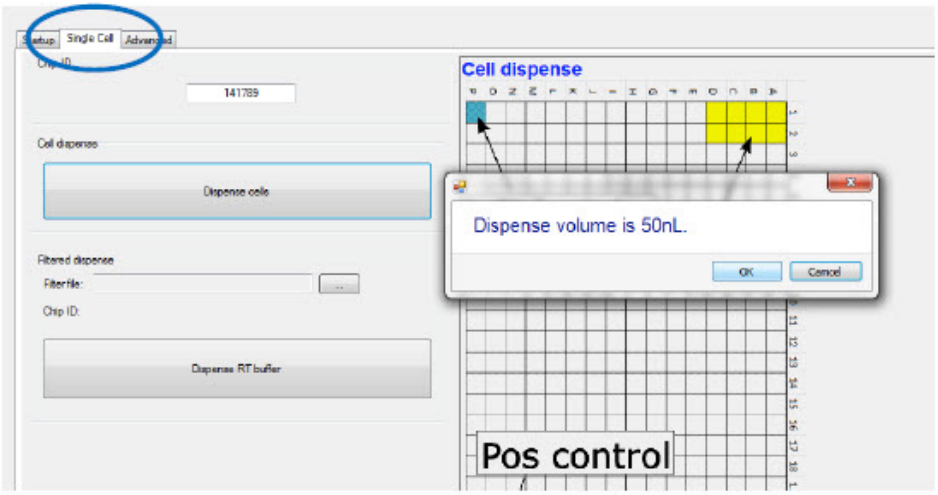

Figure 26. ICELL8 system software, Single Cell tab displaying dispense cells confirmation pop-up window.

If the humidity is not high enough, the [Dispense Samples] button will flash, and the following message will appear:

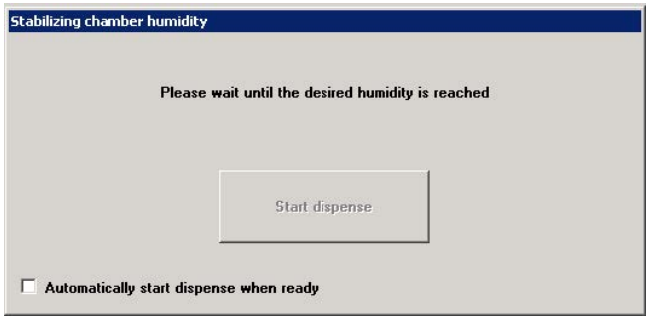

Figure 27. Stabilizing chamber humidity error pop-up.

When the humidity reaches the correct level, the [Start dispense] button turns green. Click the button to begin dispensing.

Figure 28. [Start dispense] button prior to dispense.

5. Blot and Seal SmartChip

1. After dispensing is complete, promptly blot the chip for 2 seconds.
   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 SmartChip 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 seconds 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 imaging film.

NOTE: Small bubbles on the periphery of the chip will not cause problems but bubbles on top of nanowells will.

6. Image the sample

1. Centrifuge.
   a. Place your chip on the centrifuge tray.
   b. Counterbalance with a second chip.
   c. Centrifuge at 3220 RCF (x *g*) for 15 minutes at 20–25 °C.
2. Take the chip to the ICELL8 Imaging System.
3. Image the chip. See the *ICELL8 Imaging System User Manual* for complete instructions on imaging the sample.
4. Freeze the chip to maintain RNA integrity.
5. Acquire the filter file from imaging. See the *ICELL8 Imaging System User Manual* for complete instructions on imaging the sample.

D. Prepare the source plate for RT mix.

1. Dispense RT mix into 384-well plate

Refer to the plate map shown on the Single Cell tab or shown below:

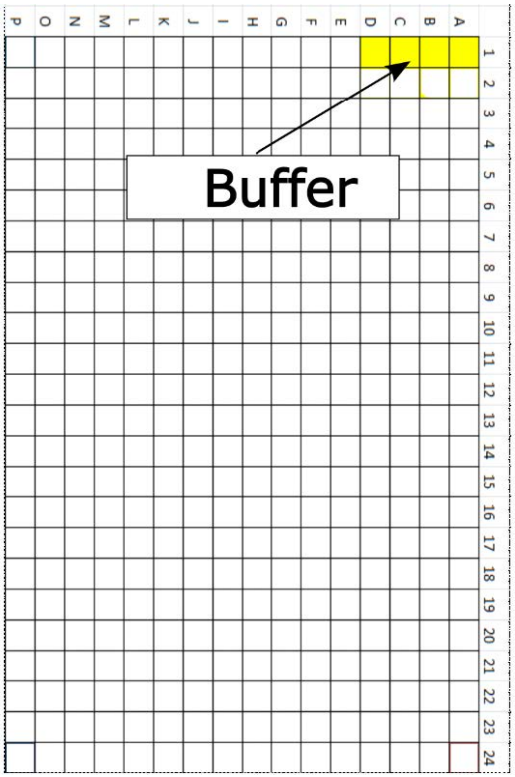

Figure 29. 384-well plate map. Buffer wells are indicated as Column 1, Rows A-D.

2. Dispense RT mix into the ICELL8 chip

1. Thaw the chip removed from the freezer. Leave at room temperature for at least 20 minutes.
2. Place the 384-well plate containing RT mix onto the plate nest with the A1 position in the top, right corner.
3. Close the environmental chamber doors.

IMPORTANT! Do not open the door of the Stage Module while the MSND 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.

IMPORTANT! Do not touch the barcode reader while the MSND is dispensing. Operating the reader while the system is dispensing could interfere with the instrument run.

4. Click the `Single Cell` tab in the software (if it is not already displayed).

5. Load the filter file. Click the [Browse] button ... to find and insert the filter file. The Chip ID will be automatically filled in.

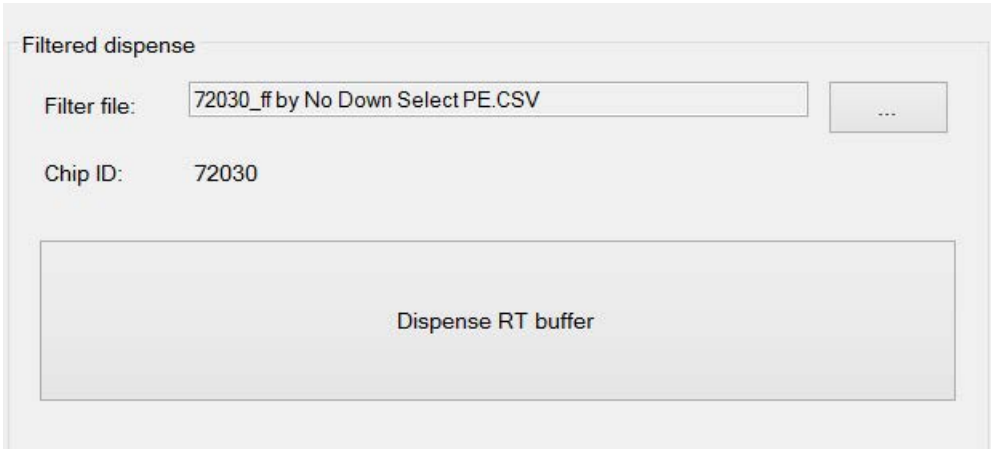

Figure 30. *Filtered dispense* window

6. Click the [Dispense RT buffer] button. The software confirms the dispense volume that will be used (see following figure). Click [OK] to dispense.

   The MSND will start dispensing 50 nl of RT buffer to the appropriate nanowells.

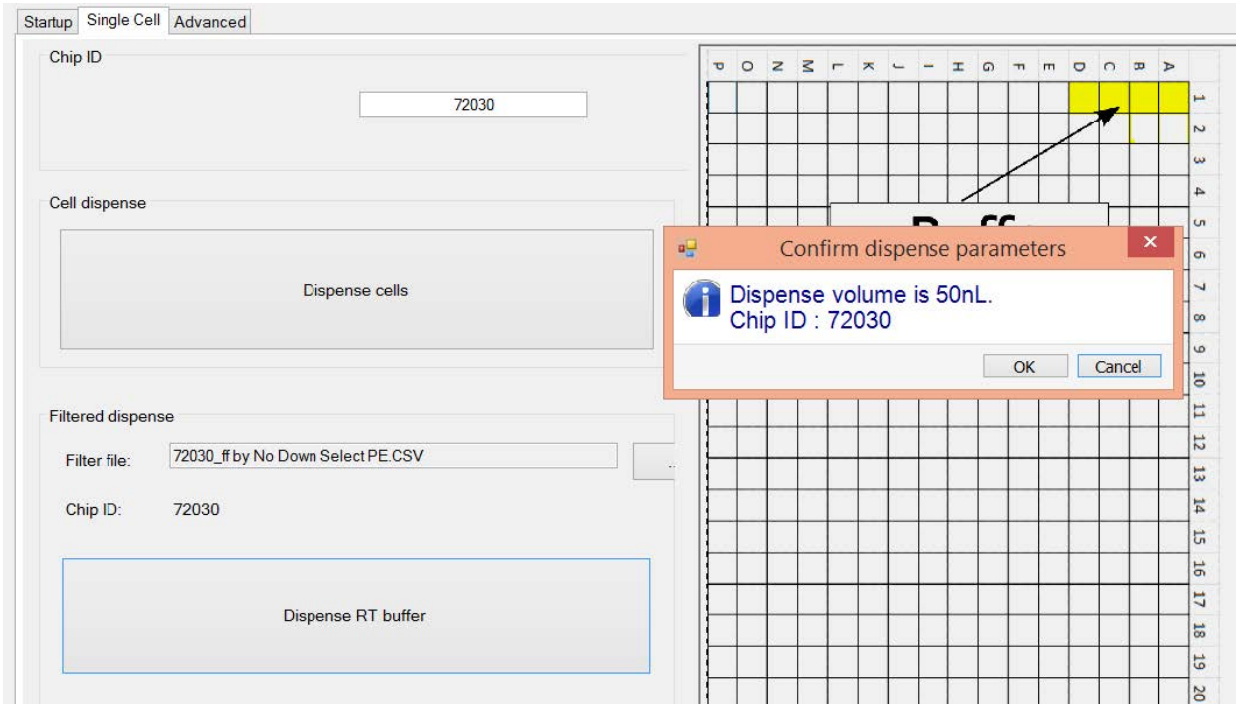

Figure 31. The *Confirm dispense parameters* pop-up window before dispense of RT buffer initiates.

7. After dispensing is complete, promptly blot the chip for 2 seconds.

8. Quickly seal the loaded chip with imaging film.

NOTE: Small bubbles on the periphery of the chip will not cause problems but bubbles on top of nanowells will.

3. Run your PCR reactions on the ICELL8 Thermal Cycler.

4. Clean the MSND.

1. After each dispensing, remove the 384-well plate from the plate nest and properly dispose of it.
2. Inspect the dispensing platform for any debris and wipe down with 70% isopropanol alcohol.
3. After the final dispense of the day, perform the Tip Clean procedure. See "Run the Tip Clean Procedure" (see Section III.A)

IV. Maintenance and Troubleshooting

A. Maintenance

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

1. Daily Maintenance

Daily maintenance procedures help ensure optimal instrument operation and prevent problems. They are described in "Prepare the MSND" (Section III.A.)

2. Complete Shutdown Procedure

Follow these instructions to completely shut down the MSND if the instrument will not be used more than a week.

1. Visually inspect the dispensing platform for any debris and wipe down with 70% isopropanol alcohol.
2. Perform the Tip Clean Procedure. See "Run the Tip Clean Procedure" (see Section III.A)
3. Exit the ICELL8 system software by clicking the Close button () at the top right of the window.
4. Turn off the peristaltic pump control box using the switch on the back-left side of the box.
5. Turn off the fluidic module using the power switch on the rear of the box.
6. Power down the computer that controls the MSND.
7. Remove all ICELL8 nanowell chips and source plates from the Stage Module and clean the dispensing platform of any debris.
8. Empty the waste container, rinse, and allow it to dry.

3. Clean the Humidifier

Empty and clean the humidifier weekly.

1. Rinse the humidifier chamber with a 1:10 dilution of commercial bleach (0.6% sodium hypochlorite).
2. Rinse 3 times with deionized filtered water.
3. Let the humidifier dry.
4. Refill with deionized filtered water.

4. Common Replacement Parts

- The external fuse (installed in the power entry module): 5 x 20 mm 5A Time Lag (Slo-Blo).
- Replace Wash tubing in the peristaltic pump box with Flexelene tubing (Eldon James Company P/N FX1-2W).

5. Annual Preventive Maintenance

Have the MSND examined and calibrated every year by a Takara Bio Service Engineer.

B. Troubleshooting

If the ICELL8 system software does not respond as desired or a warning is displayed, please attempt to rectify the problem using the table below. If you cannot solve the problem, contact Takara Bio Technical Support.

Table III. Problem: Hanging drop, drop dispersion, or improper dispensing

Possible Cause	Solution
Trapped air in the system fluidics	A large volume of air in the tube will act like a spring. When the microsolenoid valve is actuated, the air will absorb the pulse, leaving a drop hanging on the end of the tip. Inspect the tube between the tip and the 2 x 6 manifold (where not covered). Look for air or bubbles within the tube. If air is found within the tube, move the tips to the wash position and prime the system by clicking the [Daily Warmup] button.
Faulty tip connection	Inspect the slip fits on the tips and on the 2 x 6 manifold. The tubing must fit snugly to the stainless-steel tube. If the tube can be removed easily, cut off a small section of the tube and reseat the tube.
Crimped fluidic tubing	Inspect tubing for crimps or bends. Remove crimped/bent sections if feasible or replace the harness.
Plugged tip	Back-flush tips by clicking the [Tip Clean] button in the ICELL8 system software. If the tip is plugged with a soluble material, aspirating and dispensing reagent capable of dissolving the material may clear the blockage. If these measures fail, it may be necessary to replace the tip, or the blockage could be upstream of the tip. Contact Takara Bio Technical Support.

Leaking inlet valve	Let the system sit idle for 5 minutes and inspect the dispensing tips. If there are water bubbles at any of the tips, the corresponding solenoid is leaking. Contact Takara Bio Technical Support.
Incompatible buffers and samples	Extremes of fluidic properties such as viscosity may result in poor performance, contact Takara Bio Technical Support.
Tubing in pump box is cracked	Replace existing Wash tubing with Flexelene tubing (Eldon James Company P/N FX1-2W).

Table IV. Problem: Low Pressure error described

Possible Cause	Solution
Pressure too low	Check that the helium supply regulator is set to 30 psi (2 bar) maximum for a standard system.

Table V. Problem: Dispensing head does not home

Possible Cause	Solution
No communication with instrument	• Cycle the power on the fluidic module and restart the computer. • Contact Takara Bio Technical Support.

Table VI. Problem: Low or partial dispenses

Possible Cause	Solution
Microsolenoid valve failure	Contact Takara Bio Technical Support.
Relative height of system components	The relative height of the pressure reservoir, Stage Module and fluidic module must be the same as when the system was installed. If you change the height of any component and are seeing low or partial dispenses, contact Takara Bio Technical Support.
Crimped tubing	See Hanging Drop above.
Pressure too low	• Check that the helium supply regulator is set to 30 psi (2 bar) maximum for a standard system. • Contact Takara Bio Technical Support.

Table VII. Problem: Apparent low sample concentration

Possible Cause	Solution
Syringe thumb screw loose	Tighten the syringe thumbscrew until finger tight. The thumbscrew is located at the bottom of the syringe in the fluidic module.
Tip plugged	See action for Hanging drop, above.

Air bubble in syringe path	Prime the syringe path by performing the Daily Warmup.
Microsolenoid valve leaky	See action for Hanging Drop / Leaking Inlet Microsolenoid Valve, above.
Syringe valve blocked or leaky	See action for Hanging Drop / Leaking Inlet Microsolenoid Valve, above.
Low liquid level in pressure reservoir	Check the level of system liquid in the pressure reservoir. Make sure that the end of the tubing is submerged in the water. Add deionized (DI), degassed water as needed (see Section III.A).

Table VIII. Problem: System stalls because syringe does not move

Possible Cause	Solution
No power	Inspect power cables and connections.
Syringe not initialized	Cycle the power on the fluidic module and restart the computer.
Obstruction	Verify that the syringes are not obstructed.

Table IX. Problem: Persistent soft clicking (digital pressure regulator in the fluidic module has a soft click during normal operation to maintain pressure)

Possible Cause	Solution
Helium leak in system	Place soapy water around the following gas connections in the system: • 5-port cap connection to pressure reservoir. • All 5-port cap ports. • Inlet and outlet of pressure relief valve. • Gas outlet connection at the back of the fluidic module. • Gas inlet connection on back of fluidic module. • Helium tank connections.
	Check for holes in the tubing from the gas outlet of the fluidic module into the helium input ports (normally the green and blue ports of the pressure reservoir).
	Check that the O-Ring underneath the 5-port cap is seated correctly.
	Ensure that the stopcock for the vent port on the pressure reservoir is in the closed position (the black port is closed on a standard pressure bottle).
	Ensure that the ferrule orientations for the helium input ports on the pressure reservoir are correct.

Table X. Problem: Loud digital pressure regulator chattering

Possible Cause	Solution
Gas path blocked at the pressure reservoir inlet	Ensure that the stopcock for the standard helium input port on the pressure reservoir is in the open position (this is the blue port on a standard reservoir bottle and should be in the vertical position). Verify that the input pressure from the helium tank regulators is in the appropriate range and not too high.

Table XI. Problem: Soft Digital Pressure Regulator clicking and fluid leak observed

Possible Cause	Solution
Fluid leak in system downstream of helium input	Locate the source of the leak. Tighten fittings and tubing at the source of the leak. Possible sources include the following: • Liquid output ports on pressure reservoir (red and yellow ports on a standard bottle). Check that the fittings are tight and that the ferrule orientations are correct. • 8-port manifold. • Hole in tubing. • Connections at the microsolenoid valve. • Anywhere in the syringe or pressure paths

Appendix: Alcohol Wash Procedure

Use this procedure to remove trapped bubbles from the syringes in the fluidic system.

You will need ~500 mL of 70% isopropanol and a clean plastic bag to hold the fluidic harness during the procedure.

1. **Open the top portion of the protective cover of the pressure reservoir, being careful not to damage the tubes coming from the lid.**
2. **Put on clean gloves.**

3. Drain the water from the pressure reservoir and replace it with isopropanol.

1. Vent the helium by closing the stopcock on the helium-in line and opening the vent stopcock.

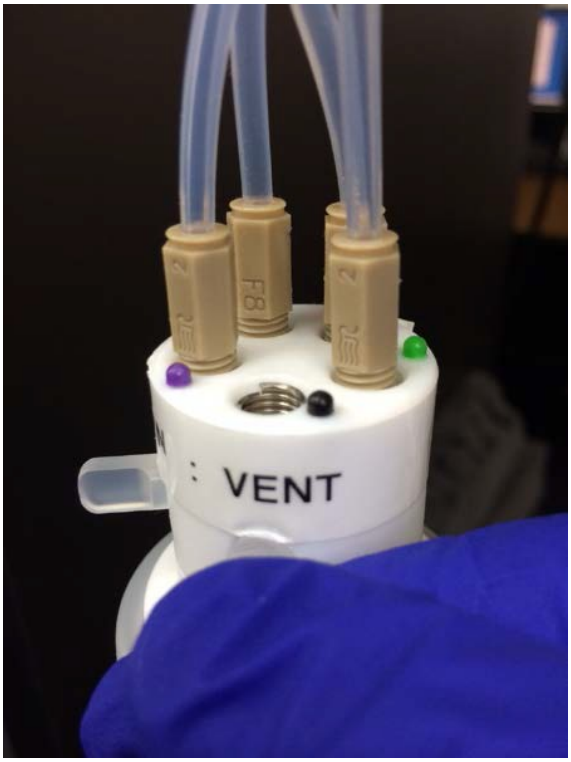

Figure 32. Illustration of the vent stopcock label.

2. Carefully remove the cap and tubing from the pressure reservoir.

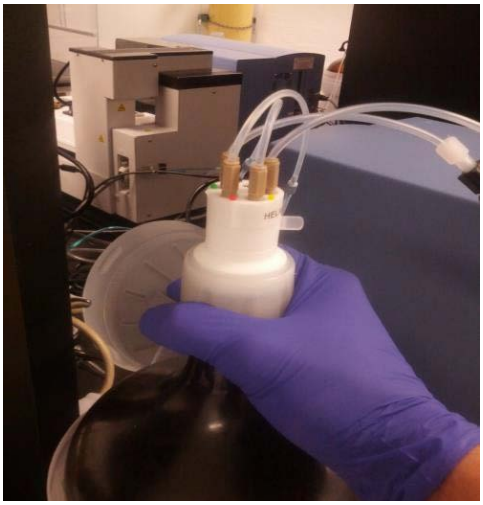

Figure 33. Removing the pressure reservoir cap unit.

3. Lift the fluidic harness, open and place the plastic bag under the harness. Insert the fluidic harness inside the bag and place on the bench.

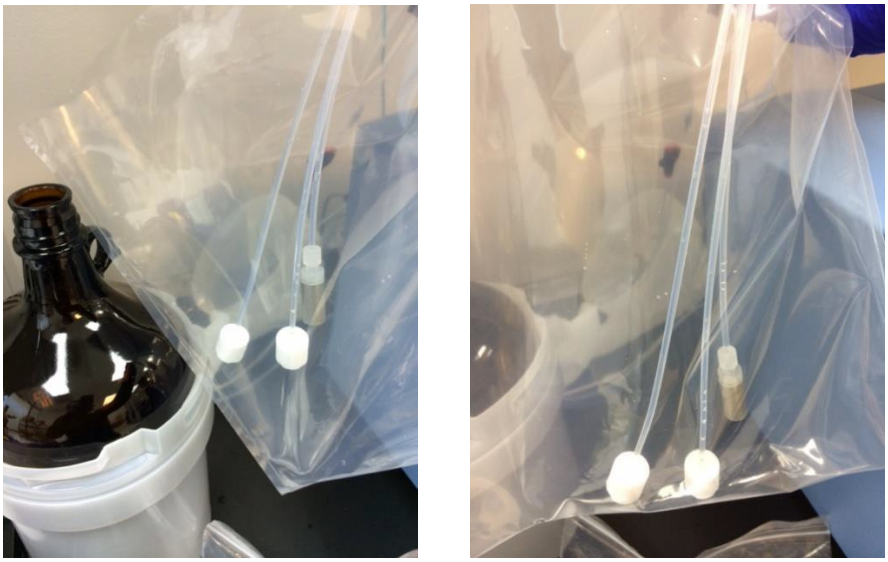

Figure 34. Inserting the fluidic harness in its plastic bag

4. Empty the water from the pressure reservoir.
5. Fill the bottle with 500 mL of 70% isopropanol.
6. Reattach the cap and replace the tubes in the reservoir. Avoid touching the tubes when inserting into the bottom.
7. Reattach the top of the protective cover.
8. Close the system by opening the stopcock on the helium-in line and closing the vent top cock.
9. Reattach the protective cover.

4. Prime the system with 70% isopropanol by running the first portion of the Daily Warmup procedure.

Section III.A, Step 5. Click the [System Prime] button.

5. Run the Daily Warmup procedure.

6. Refill the reservoir with water.

1. When the Daily Warmup is complete, depressurize the reservoir.
2. Follow the instructions in step 3 above to remove fluidic harness. The same plastic bag can be used for this step.
3. After the fluidic harness has been removed, drain the remaining isopropanol from the reservoir to a waste container.

4. Rinse the reservoir thoroughly with deionized filtered (0.2 µm) water.
5. Fill the bottle with deionized filtered water to the top of the bottom section of the protective cover.
6. Let the reservoir liquid degas for 30 minutes. You should see helium bubbling through the water during this period.
7. Prime the system twice, following the instructions in step 4 above.

7. Repeat the Daily Warmup procedure once more.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com	web: takarabio.com
e-mail: ordersUS@takarabio.com	e-mail: technical_support@takarabio.com

Notice to Purchaser

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements

This document has been reviewed and approved by the Quality Department.