NUCLEIC ACID PURIFICATION
PURE AND SIMPLE®

PROTOCOL

Ionic® Cells to Pure DNA Kit

REV 1.0

PART #: 33002

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PURIGEN® BIOSYSTEMS

Table of Content

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Support

For technical information or advice, please contact Purigen Biosystems Support at any time.

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Safety Recommendations

For all procedures in this document, the use of appropriate personal protective equipment (PPE) is strongly recommended. Disposable gloves should always be worn when handling samples, reagents, fluidic chips, and any other materials that may come into contact with samples. Gloves should be changed immediately after any contact with the sample.

Inappropriate use of an Ionic® Purification System may cause personal injury or irreparable damage to the instrument.

- Only trained personnel should operate the Ionic system following Purigen Biosystems published methods
- All operators should review and be familiar with the **Ionic Purification System User Guide**
- Only Purigen Biosystems qualified service engineers should service the Ionic system

Damage to the Ionic system caused by inappropriate use, neglect to perform required maintenance, or performing inappropriate maintenance may void warranty or require services not covered by standard service contract terms.

- Do not move the instrument while it is in operation
- Do not unplug the instrument while it is in operation
- Do not spill liquids on any area of the instrument
- Do not use with flammable materials or in the presence of toxic fumes
- Do not use excessive force to open or close the system cover
- Use only with Purigen Biosystems Ionic Fluidic Chips and associated kits and protocols

Laboratory supervisors and/or facility managers must take the necessary precautions to ensure a safe workplace and appropriate training of personnel.

- All laboratory activities should be in accordance with all national, state, and local health and safety regulations
- Follow all applicable SDS (or MSDS) recommendations for proper handling and disposal of chemicals and reagents
- Follow all safety guidelines for use of personal protective equipment, laboratory devices, and labware established for the laboratory where the instrument is used

Kit Contents

TABLE 1: Contents provided in the Ionic® Cells to Pure DNA

Container	Item Name	Description	Volume	Quantity per Kit
-20°C Reagents Box	**Lysis Buffer 1**	Lysis Buffer 1	1.2 mL	6
-20°C Reagents Box	**RNase A**	RNase Reagent	240.0 µL	1
-20°C Reagents Box	**Proteinase K**	Proteinase K Reagent	480.0 µL	1
RT Reagents Box	**1 - Extraction Buffer**	Extraction Buffer	12.0 mL	1
RT Reagents Box	**2 - Anodic Buffer**	Anodic Buffer	12.0 mL	1
RT Reagents Box	**3 - Separation Buffer**	Separation Buffer	18.0 mL	1
RT Reagents Box	**4 - Neutralization Buffer**	Neutralization Buffer	12.0 mL	1
RT Reagents Box	**5 - Cathodic Buffer**	Cathodic Buffer	12.0 mL	1
RT Reagents Box	**Lysis Buffer 2**	Lysis Buffer 2	7.2 mL	1
RT Reagents Box	**Sample Buffer**	Sample Buffer	1.8 mL	1
Fluidic Chip Set Box	**Purigen Ionic Fluidic Chip**	Fluidic Chips	N/A	6

TABLE 2: Reagents provided by the user

User-supplied Reagents	For Lysate Preparation	For Purification
Media containing cells	✓	
Dulbecco's Phosphate Buffered Saline (D-PBS) (without Ca++ and Mg++)	✓	

TABLE 3: Equipment provided by the user

User-supplied Equipment	For Lysate Preparation	For Purification
12-column reservoir (Agilent 204365)		✓
P200 multichannel pipette		✓
P200 single channel pipette	✓	
P200 wide bore tips	✓	
P1000 single channel pipette	✓	
P20 single channel pipette	✓	✓
Microcentrifuge	✓	
Programmable ThermoMixer	✓	
Vortex mixer (adjustable speed)	✓	✓

TABLE 4: Labware provided by the user

User-supplied Labware	For Lysate Preparation	For Purification
DNA LoBind Tube, 1.5 mL (Eppendorf 22431021)	✓	✓
Optional: **DNA LoBind Tube, 2.0 mL** (Eppendorf 22431048)	✓	
Optional: **DNA LoBind Plate, 96-well** (Eppendorf 951032000)		✓

Preparation of Cells

This protocol supports the extraction of DNA from cell samples ranging from 50,000 cells to 5 million cells*. The protocol supports extraction from White Blood Cells (WBCs) and Peripheral Blood Mononuclear Cells (PBMCs) isolated from blood in addition to cultured and/or flow-sorted cells.

**Cell counts as low as 10 cells may be achieved with alternate protocols. Contact support@purigenbio.com to learn more.*

Fresh Cells

1. Harvest and count cells.
2. Proceed with **Prepare Samples for Lysis.**

Frozen Cells

- If cells are already present as a dry pellet that has not been washed or are suspended in frozen media/PBS:
    1. Resuspend the cells in 175 µL 1X PBS (Dulbecco's Phosphate Buffered Saline [D-PBS] without Ca++ and Mg++) and gently pipette up and down 5X.
    2. Spin down at 500 RCF for 5 minutes.
    3. Carefully remove supernatant without disturbing pellet, making sure to leave no more than 5 µL of PBS behind.
    4. Proceed with **Prepare Samples for Lysis Steps 1–5** (skip Step 6)

- If the cells are in a dry pellet that has been washed with PBS, then
    - For 50,000 – 1,000,000 cells:
        1. Proceed directly to **Prepare Samples for Lysis**
    - For cell above 1,000,000:
        1. Spin down at 20,000 RCF for 1 minute.
        2. Carefully remove the media without disturbing the pellet. Remove as much volume as possible, leaving no more than 5 µL in the tube. Use a smaller pipette tip if necessary.
        3. Proceed with **Prepare Samples for Lysis Steps**

NOTE
Without disturbing the cells, remove the maximum amount of buffer/wash to minimize carry forward of ions.

TABLE 5: Table name

Cell Number	Desired or Recommended Condition
50,000 cultured cells	In 10 µL media
50,000 PBMC and WBC	In 10 µL PBS
Above 50,000	Pelleted

Lysate Preparation – Single Tube Format

NOTE
It is assumed that **standard laboratory equipment** such as disposable gloves or a refrigerator and freezer are available for use as needed.

Prepare Samples for Lysis

1. Create a **Lysis Program on the thermomixer** with the following parameters:
   - Incubate at 56°C for 4 hrs at 1400 RPM
2. Thaw **Lysis Buffer 1** at 56°C for 10 minutes. Briefly vortex and centrifuge **Lysis Buffer 1** to collect any evaporate.
3. Thaw **Lysis Buffer 2** at room temperature.

NOTE
The thermomixer should accommodate **1.5 mL or 2 mL** microcentrifuge tubes, depending on the tubes used for the preps. Use of a thermomixer with its lid is recommended.

IMPORTANT
It is important that **Lysis Buffer 1** is at **room temperature** before vortexing and using.

IMPORTANT
All centrifuge steps should occur at **room temperature**.

4. Place **Proteinase K** on ice.
5. Place **RNase A** on ice.

NOTE
RNase does not pipette accurately if it is below room temperature.

6. Gather thawed **Lysis Buffer 1** and **Lysis Buffer 2,** and **keep at room temperature** until proceeding to Step 7.
7. Dispense required number of cells (see **TABLE 5**) into each sample tube (Eppendorf 1.5 mL DNA LoBind Tubes) and proceed to **Lysis Procedure**.

NOTE
For cell counts >50k, it is best to pellet the cells prior to lysis. For cell counts <50k, the should be suspended in 10 µL of media for cultured cells or 10 µL PBS for WBCs/PBMCs.

Lysis Procedure

IMPORTANT

It is critical to process each sample tube individually. For one sample tube at a time, follow Step 1 (add Lysis Buffer 1) immediately with Step 2 (add Lysis Buffer 2).

1. For the first sample tube, add **110 µL Lysis Buffer 1** using a P200 pipette and pulse vortex for 5 seconds. Complete 5 seconds vortexing is critical.
2. For the first sample tube, add **110 µL Lysis Buffer 2** using a P200 pipette and pulse vortex for 5 seconds. Complete 5 seconds vortexing is critical.
3. **Repeat** steps 1-2 for each of the remaining sample tubes.
4. Add 10 µL **Proteinase K** and 5 µL **RNase A** to each sample tube.
5. Vortex all sample tubes for 5 seconds and spin briefly.
6. Incubate on thermomixer at **56°C for 4 hours at 1400 RPM**.
7. Immediately spin the samples briefly to collect any condensate from the top of the tube. If you do not plan to purify the samples within 12 hours, **freeze at -20°C**. Samples can be stored at **4°C for short term storage** (< 12 hours) or kept on ice if you are proceeding directly to **Purification** on the following page.

IMPORTANT

Sample tubes can be stored at **4°C for a maximum of 12 hours** prior to purification. Sample tubes should be stored at -20°C when stored for more than 24 hours.

NOTE

For frozen samples, thaw them to **room temperature, th**en vortex and spin briefly before proceeding with the protocol.

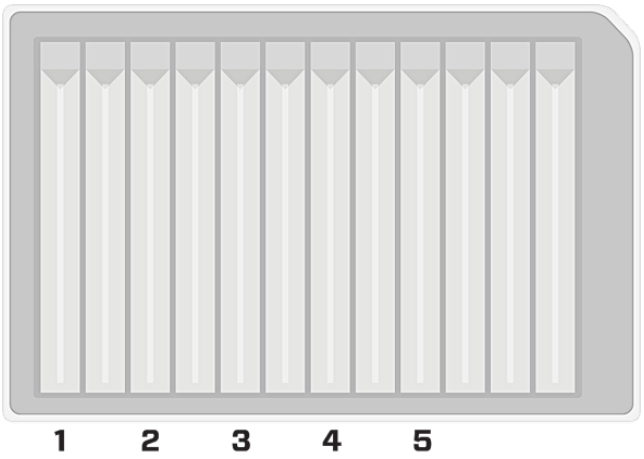
Purification

Prepare Samples and Reagents

Setup the Reagent Reservoir

1. While wearing gloves, remove the 12-column reagent reservoir from it packaging.
2. Using a permanent marker, label 5 columns of the reservoir as shown below. Separate columns 1-5 with empty columns to prevent cross contamination.

FIGURE 1: Reservoir column labels



3. Add buffers to each column according to the following table.

TABLE 6: Reservoir buffers and volumes

Reservoir Column	Buffer from Kit		Volume
1	**Extraction Buffer**	**(1)**	2.0 mL
2	**Anodic Buffer**	**(2)**	2.0 mL
3	**Separation Buffer**	**(3)**	2.5 mL
4	**Neutralization Buffer**	**(4)**	2.0 mL
5	**Cathodic Buffer**	**(5)**	2.0 mL

4. Ensure that the bottom surface of each column is completely covered with reagent by gently tapping or tilting the reservoir.

Thaw Samples (if previously frozen)

1. If samples have been stored in a freezer, thaw samples to room temperature.
2. Before loading on to the fluidic chip, vortex all samples for 3 seconds and centrifuge briefly.

IMPORTANT

All samples **must be at room temperature** prior to loading the fluidic chip.

Using the Instrument

IMPORTANT

Do not relocate the instrument after installation by a Purigen Biosystems representative. A change to the alignment or level of the instrument can affect the system's performance. Please contact your Purigen Biosystems representative to relocate the instrument.

1. Turn on the system using the power switch at the rear of the instrument.
2. Ensure the instrument cover is free of any obstructions.
3. Press **Start** on the Start screen.

FIGURE 2: Start screen

Create or Select User Profile

1. If you have not already created a **User Profile**, create a profile by pressing the ⊕ button at the bottom of the touch screen
2. Use the touch screen keyboard to enter the desired **User Name** and press **Save**. After saving the new user profile, it will be visible in the **Select User** menu.

FIGURE 3: Create New User screen

NOTE
Select the ✕ icon when creating a new user to clear existing text.

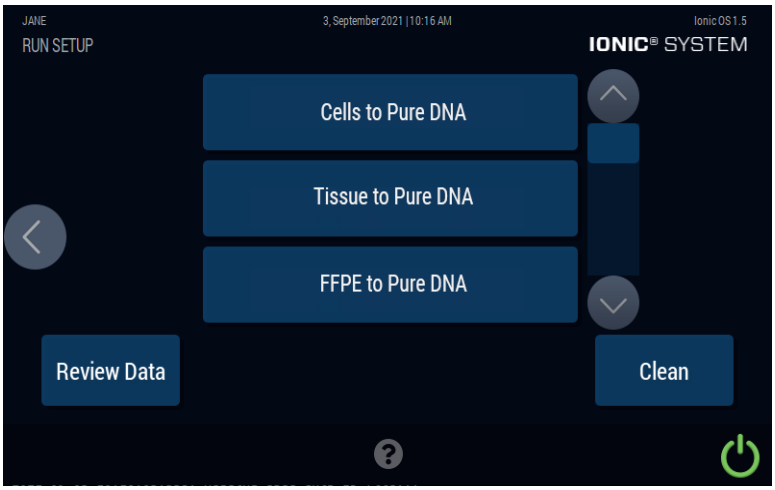
NOTE
After saving the new user profile, it will be visible in the **Select User** menu in the future.

3. Select the user profile you just created or a previously created profile to go on to the next step.

Setup a Run

1. Ensure that no objects are resting on the instrument cover and that the cover is completely free of any obstructions while the system is in use.
2. **Select a protocol.** This will cause the instrument cover to retract into the system, exposing the fluidic chip holder. The screen will display a warning that the cover will be opening.

FIGURE 4: Run Setup screen



Load the Fluidic Chip

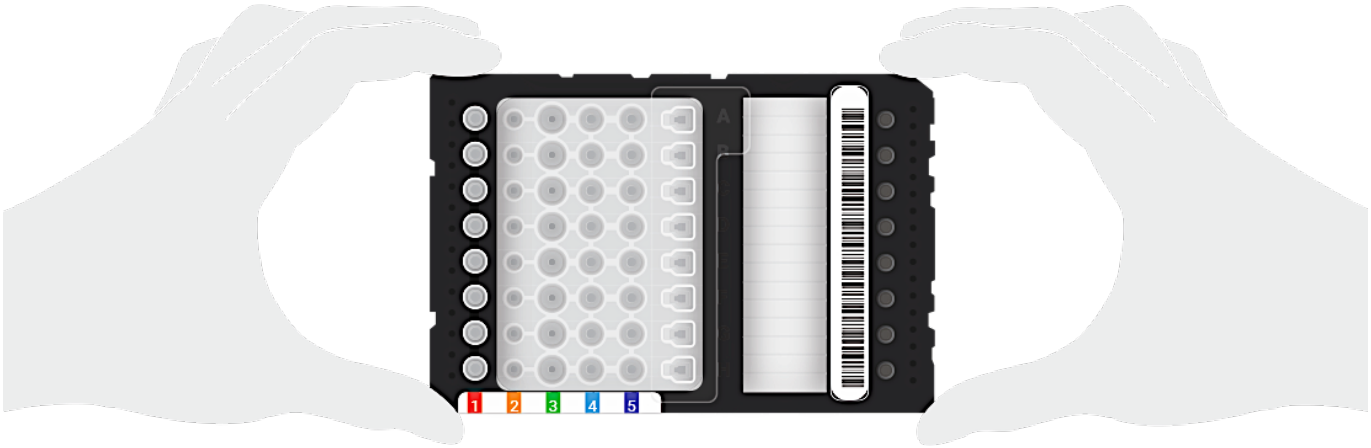
1. Position samples and reagent reservoir near the instrument.
2. While wearing gloves, remove a fluidic chip from its packaging.

IMPORTANT
Use the Ionic Tissue Chips provided with your extraction kit. **DD NOT** use chips provided with other kits.

CAUTION
Do not remove a fluidic chip until you are ready to begin a Run. Always handle the fluidic chip from the side skirting – see image below. Contact with the bottom of the chip may cause damage.

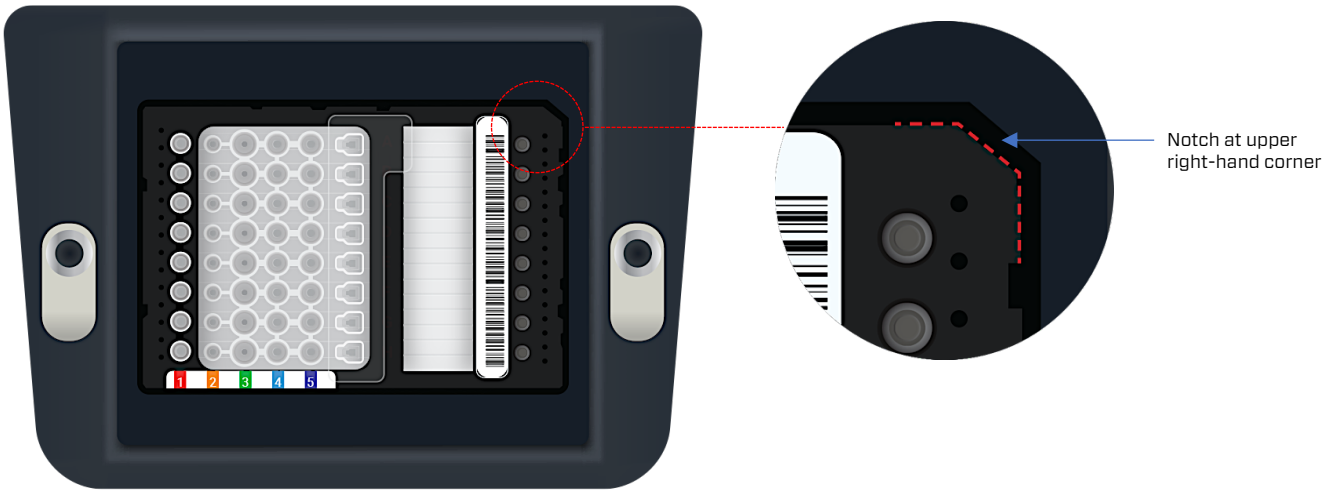
IMPORTANT
If samples have been frozen prior to use, make sure that they are completely thawed, vortexed, and at room temperature before loading.

FIGURE 5: Proper placement of fingertips when holding a fluidic chip

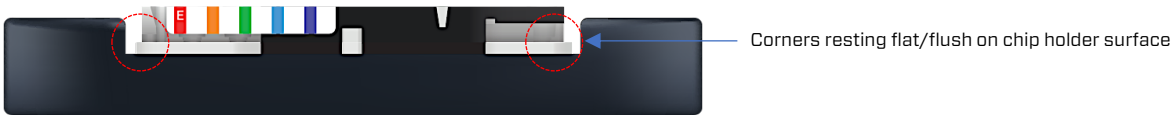


3. Identify the notched corner of the chip and orient the chip such that the notch is at the upper right-hand corner (see **FIGURE 6**).
4. Gently apply pressure to all four corners of the chip simultaneously to confirm that the chip is fully seated in the holder.

FIGURE 6: Proper orientation and placement of chip on the instrument chip holder



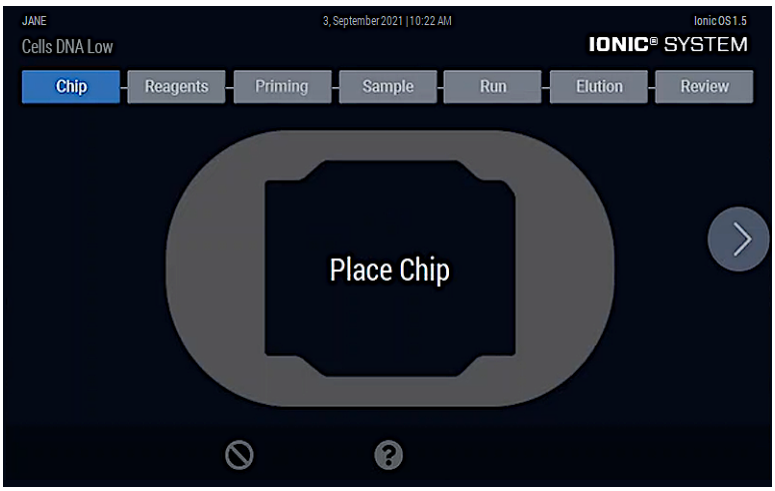
Top-down view of fluidic chip on instrument



Profile view of fluidic chip flat/flush on instrument

5. On the instrument's touch screen, press the **Arrow** button shown on the right side of the screen.

FIGURE 7: Insert Chip screen



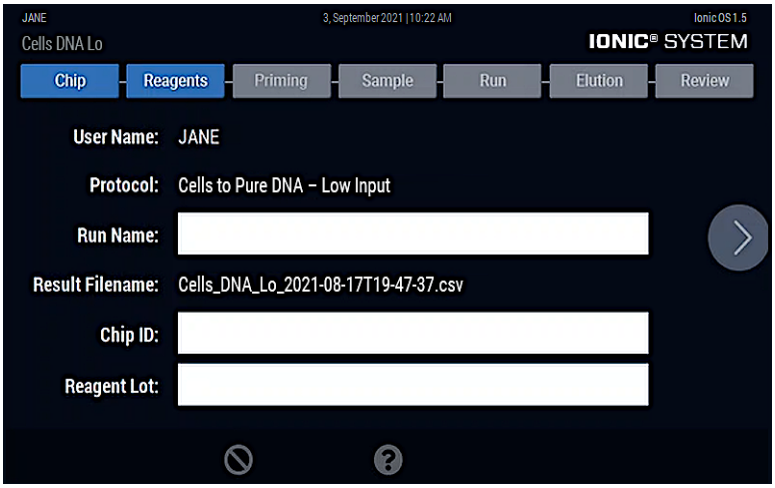
6. The **Reagents** screen is displayed.

Load Reagents

1. Using the touchscreen or a barcode reader, enter the **Run Name, Chip ID**, and **Reagent Lot** values and press the arrow button on the right side of the screen.

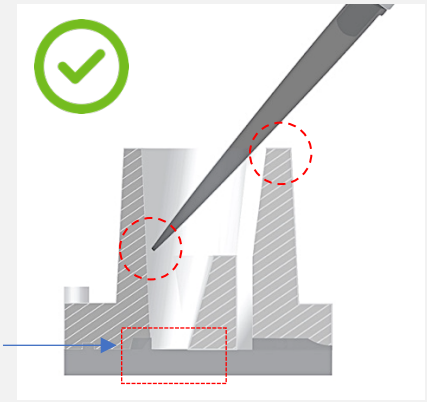
NOTE
The **Reagent Lot** number can be found on the label of the reagent box from the kit. The **Chip ID** is located on a label on the top surface of the fluidic chip. The barcodes of these labels can be scanned into the software using a handheld barcode reader plugged into the USB port of the Ionic system.

FIGURE 8: Reagent screen – **Step 1**



IMPORTANT
When adding buffers to the chip, visually check to make sure that all tips contain the same volume of reagent and **depress the pipette plunger to the first stop only.** Change tips after each dispense.

IMPORTANT
When adding buffers to the chip, visually check to make sure that all tips contain the same volume of reagent. Angle the pipette such that the upper portion of the **pipette tip rests on the right-hand wall of the chip well while the end of the tip is touching the left-hand wall of the chip well** so that the pipette is at a 45 angle relative to the chip surface. Avoid contact with the bottom of the chip-well when dispensing**. Smoothly depress the pipette plunger to the first stop only.** Change tips after each dispense.



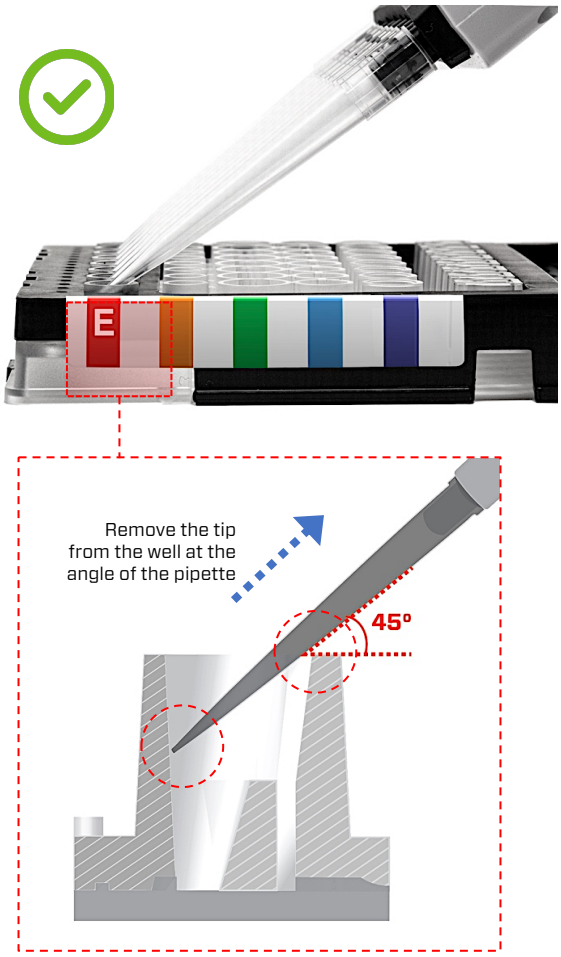
Pipetting Technique for Dispensing Extraction Buffer into Column 1

Below are instructions on how to properly dispense the Extraction Buffer into the Column 1 wells. Please follow this technique to prevent errors in the quality of the extraction.

Correct Pipetting Technique

The end of the pipette tips should rest on the inner left wall of the wells 30–50% down at a 45° angle relative to the surface of the chip.

FIGURE 9A: Correct pipetting technique and position

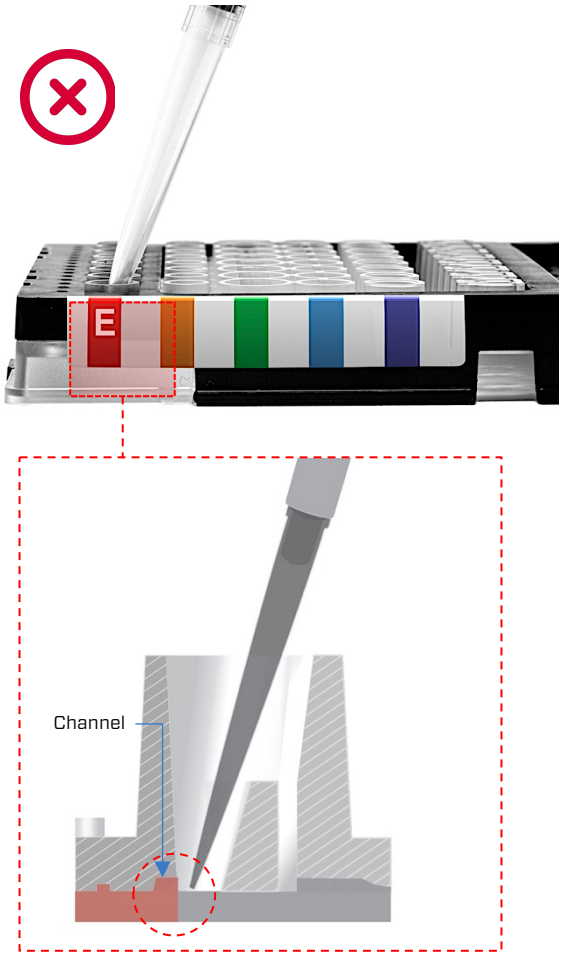


- Hold pipette approximately at 45° angle relative to the surface of the top of well.
- Tip of pipette should rest against left wall of the well.
- Rest the barrel of the tip against the upper-right side
- Continuously and smoothly dispense the fluid into the well to avoid air bubbles. **Stop at the first stop – do not fully depress the plunger.**
- Remove the tips from the wells immediately after dispensing the buffer.

Incorrect Pipetting Technique

The pipette tips should not be positioned below 50% of the well depth or at the bottom of the wells when dispensing to avoid fluid being injected directly into the channel.

FIGURE 9B: Incorrect pipetting technique and position

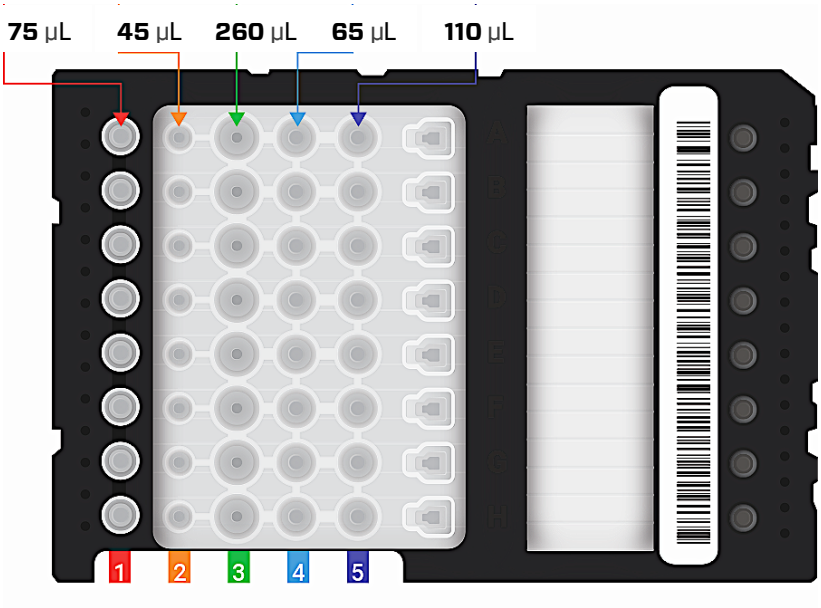


- Do not insert the tip to the bottom of the well to avoid injecting fluid directly into the channel

NOTE
If using a P200 pipette, the Separation Buffer should be added using two pipette transfers with a clean tip for each transfer.

2. Using a P200 multichannel pipette, add buffers from the reagent reservoir, working left to right (1 to 5), to the corresponding wells of the fluidic chip at the volumes displayed on the screen. **Be sure to insert the pipette tip no more than 50% of the way** into the reagent wells of the chip when dispensing.

FIGURE 10: Buffer dispense volumes



Prime the Fluidic Chip

1. After loading the reagents, ensure the chip holder is clear of any objects except the chip and press the arrow button on the right side of the screen. This instructs the instrument to close the cover and begin to prime the chip.

IMPORTANT
A priming cycle takes approximately 3 minutes. **Samples should be loaded within 5 minutes of priming completion**.

2. After the priming cycle completes, a message is displayed asking you to confirm if you have vortexed the sample as instructed in previous steps. If samples were previously frozen, vortex lysates for 3 seconds and centrifuge briefly. Press **OK** once the vortex is complete.

NOTE
The vortex step is only required for previously frozen samples.

FIGURE 11: Priming Message screen

3. Press the **Arrow** button on the right side of the screen. The instrument cover will open.

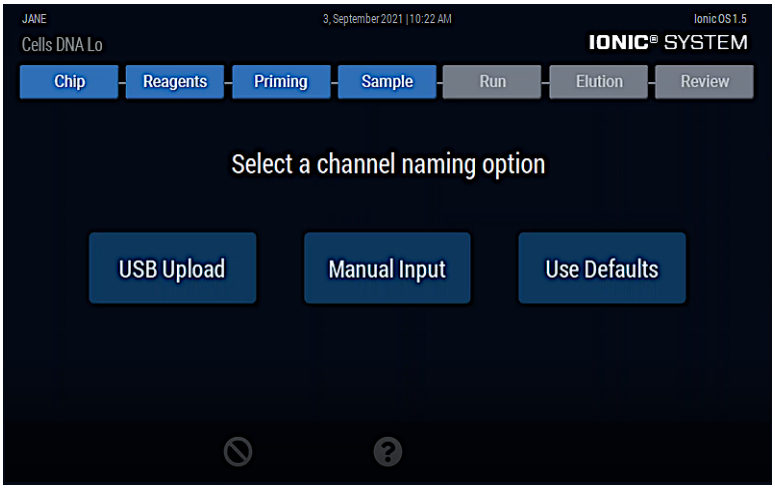
Load Samples on the Fluidic Chip

1. In the **Select a channel naming option** screen, press **USB Upload, Manual Input**, or **Use Defaults**.

> **NOTE**
> For this guide, we will be using the **Manual Input** option. For more information on use of the other options, see the Ionic system User Guide.

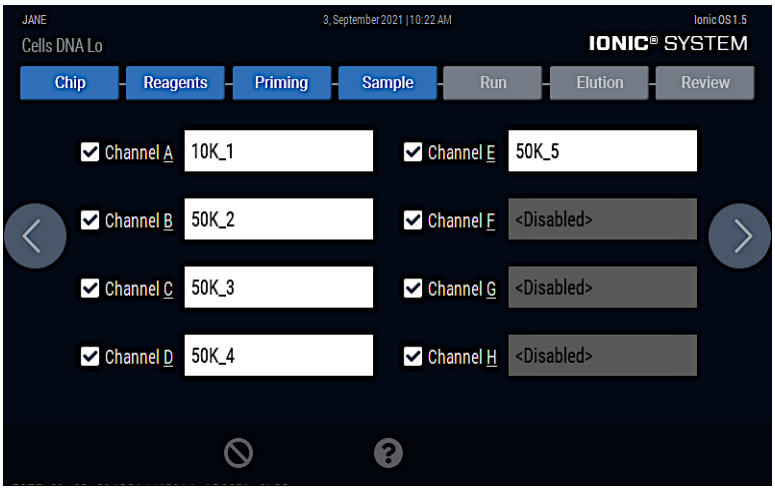
> **NOTE**
> If available, barcode information can be scanned into the software using a handheld barcode reader plugged into the USB port of the Ionic system.

FIGURE 12: Sample screen – **Step 1**



2. Using the touchscreen, manually enter custom sample names and press the **Arrow** button on the right side of the screen to continue.

FIGURE 13: Sample screen – **Step 2**

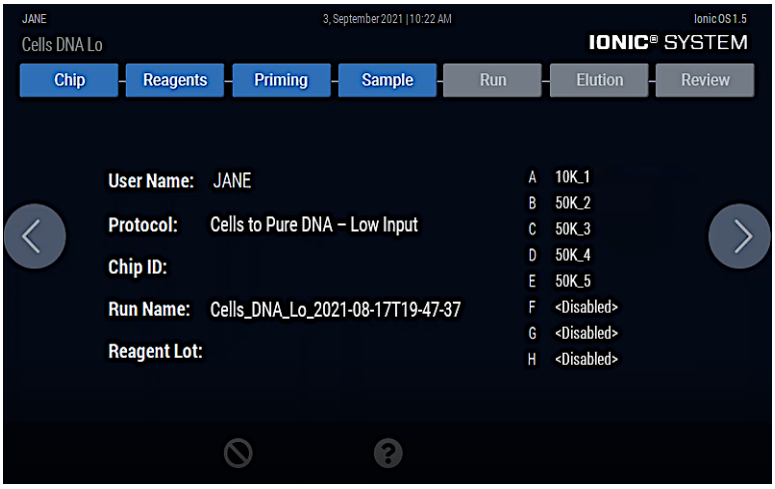


IMPORTANT

Deselect the checkbox next to a Channel if it will not be used during the run.

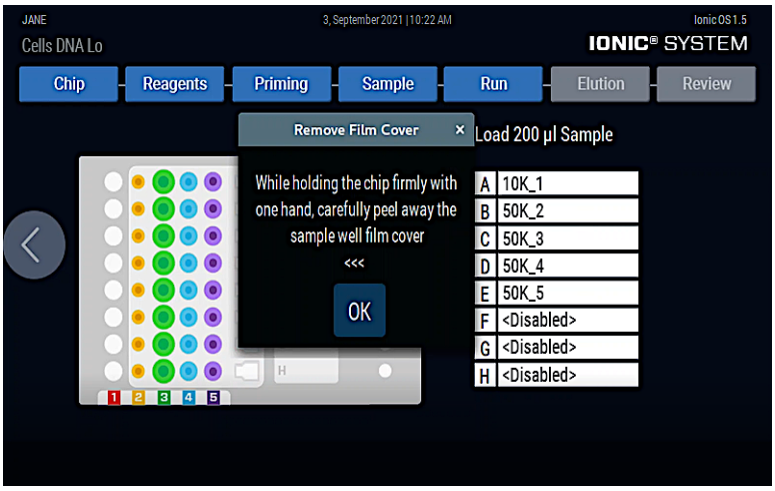
3. The **Review** screen is displayed. Verify sample names and then press the **Arrow** button on the right side of the screen to continue or press the left **Arrow** to edit the entries.

FIGURE 14: Sample screen – **Step 3**



4. A message is displayed instructing you to hold the chip firmly with one hand and carefully peel away the sample well film cover. Press **OK**.

FIGURE 15: Run screen



5. While gripping the chip appropriately on the side skirt, hold the chip securely against the chip holder and carefully peel off the adhesive cover over the sample wells using the pull tab.

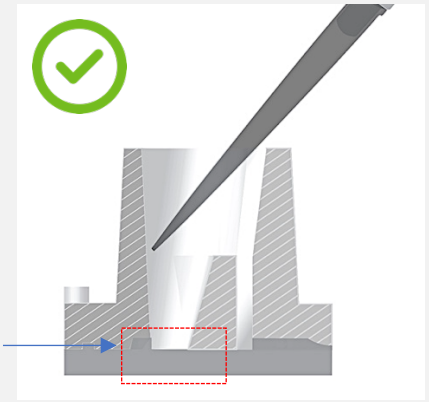
CAUTION
Avoid touching the wells or the surface or any fluids on the chip.

FIGURE 16: Remove adhesive cover

6. Using a P200 pipette, dispense **200 µL of sample** to each sample well with the tip inserted **no more than 50%** into the well. Slowly retract pipette tip from the well while dispensing to keep tip positioned at the surface of the fluid filling into the well.

CAUTION

Avoid contact with the bottom of the chip-well when dispensing.
Smoothly depress the pipette plunger to the first stop only.



Start the Run

1. Press **Begin Run**. The purification process will now start and continues for **approximately 70 minutes**.
2. After the Run is complete, ensure the top of the instrument cover is free of any obstructions and press arrow button on the right side. The instrument cover will open.

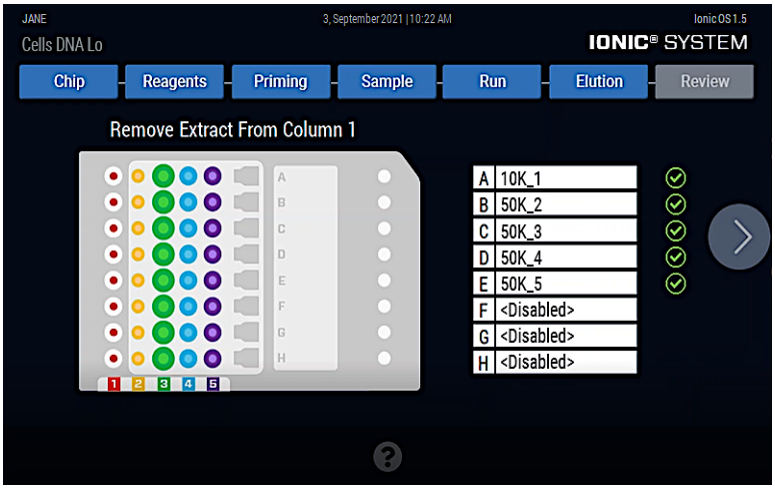
IMPORTANT
Remove samples from the chip **within 30 minutes** of Run completion.

FIGURE 17: Run Complete screen

Collect Samples

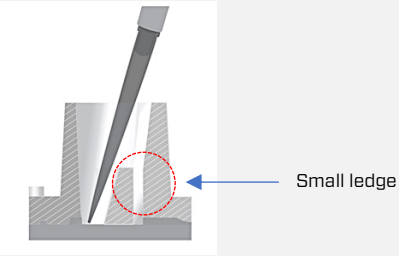
1. The **Remove Extracted DNA** screen is displayed.

FIGURE 18: Elution screen



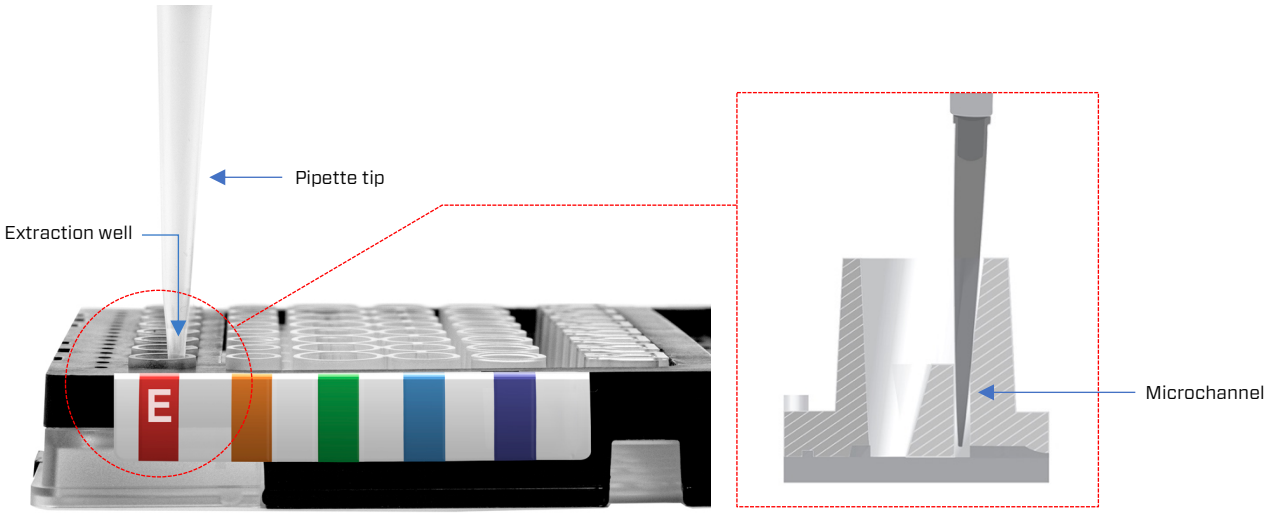
2. Set a P200 multichannel pipette to **50 µL.** Place the pipette tip at the bottom of the sidewall of the **Extraction Buffer** well (1) and **pipette mix 3 times** and transfer the extracts to an Eppendorf DNA LoBind microcentrifuge tube or DNA LoBind 96-well microplate.

NOTE
The **Extraction Buffer** well has a small ledge. Be sure to navigate the pipette tip below the small ledge to the reach the well bottom.



3. Optionally, using a P20 pipette, evacuate any remaining extraction buffer (as much as 10 µL) from the microchannel within the extraction well and add to the same plate or collection tube (see **FIGURE 20**).

FIGURE 19: Optional evacuation of microchannel within extraction well



4. Vortex collection tube or plates for **10 seconds at 2500 RPM** and centrifuge briefly before using the samples in downstream assays.

NOTE
Store extracts at **4°C for same-day use** and **20°C for long term storage**.

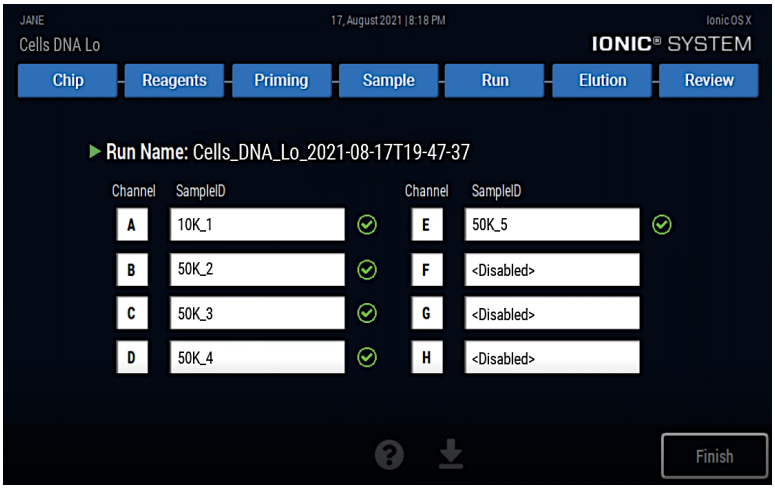
CAUTION
To ensure homogeneity for downstream assays **Step 4 must not be overlooked.**

5. Ensure the top of the instrument cover is free of any obstructions.
6. Remove the chip and press the **Arrow** button. A countdown will indicate time until cover closing.

Review Results

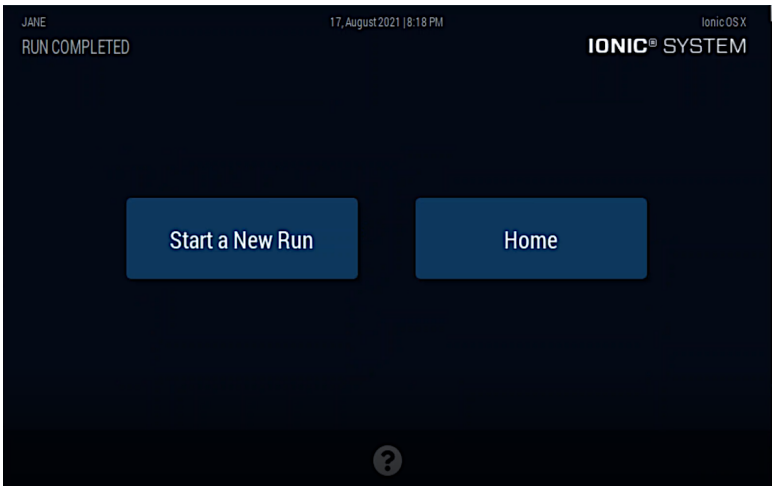
1. After the cover closes, press **Finish** to complete the Run.

FIGURE 20: Review screen



2. Press **Logout** to return to the **Choose a User** screen or press **New Run Setup** to setup another Run.

FIGURE 21: Run Completed screen



Troubleshooting

If you encounter any errors while running the instrument, follow the steps in **"Save System Logs"** and then email the log file you saved to **support@purigenbio.com**. Purigen Biosystems support will contact you within 48 hours to follow up.

Save System Logs

The **Save System Logs** Maintenance screen is used to save system log files to a USB flash drive. The system log files can be used by Purigen Biosystems to diagnose problems with the instrument.

> **NOTE**
> USB flash drive must be in a FAT32 format. It is recommended to have a minimum of 1 GB of available space on the USB flash drive.

Some cases where this function should be used:

- If the run results are not as expected
- If the self test fails
- If Purigen Biosystems Support personnel request a system log

1. Press the **Save System Logs** button to save system log files to a USB device.

FIGURE 22: Save System Logs Screen - **Step 1**

2. Insert a USB drive into the USB slot located the bottom-right on the front of the instrument.
3. Once a valid USB drive is detected, the next screen is displayed. Press **Write** to begin the transfer.

FIGURE 23: Save System Logs Screen - **Step 2**

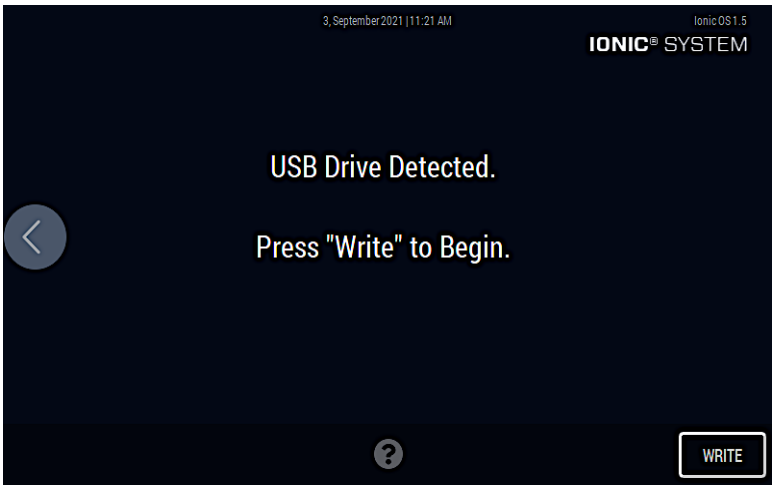


TABLE 7: Save System Logs screen

Call Out	Screen Component	Definition
1	**Write button**	A button to copy the system log to the USB drive inserted in the USB slot in the font of the instrument. The system log file is copied to the root directory of the USB drive
2	**Help icon**	Loads the Help screen

4. A status bar is displayed on the touchscreen.

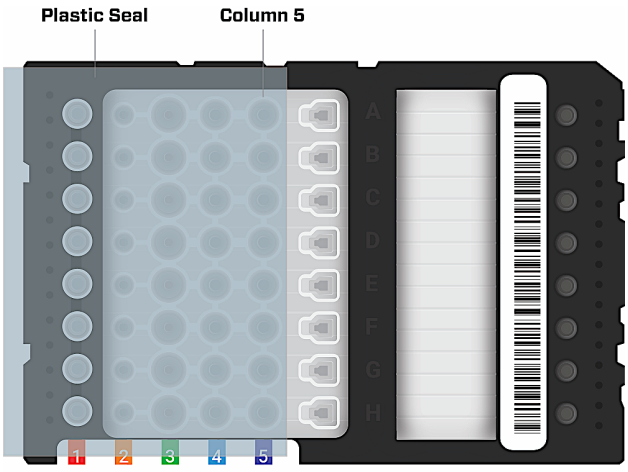
> **NOTE**
> The status bar may seem inactive for larger log files. Wait for the system log to be saved to the USB flash drive.

5. After the system log file is saved to the USB flash drive, press the button on the left side to return to the **Maintenance & Service** screen.

Sample Recovery

If the Ionic system displays a icon for one or more samples in a run, follow the steps below to recover the sample so it can be run on a new chip.

1. Before collecting unprocessed sample, attempt to recover the purified extract as described above under **"Collect Extracts of Purified Nucleic Acid."**
2. Cut a plastic plate seal in half so that it will cover all wells in Columns 1–5 of the plate, but NOT cover the sample well.

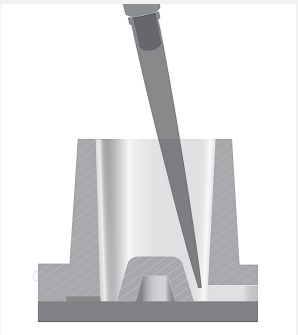


3. Cover Columns 1–5 with the plate seal while paying particular attention to ensuring a tight seal for each well in Column 5.
4. Make sure the chip is on a level surface.
5. Set 200 µL pipette to 200 µL.
6. While using your thumb/forefinger to press down and reinforce the seal on the well in Column 5, aspirate **slowly** from the bottom right side of the Sample Well where the well and channel meet (see image). Aspirating from the bottom right side of the sample well allows fluid to be collected from the sample well AND the channel to the right of it. Conversely, fluid in the channel to the left of the sample well should be avoided. Ensuring a proper seal on the well in Column 5 will help to minimize the amount of fluid (Cathodic Buffer) that is aspirated from the channel on the left side of the sample well.

NOTE

While using your thumb/forefinger to press down and reinforce the seal on the well in Column 5, aspirate **slowly** from the bottom right side of the Sample Well where the well and channel meet.

The pipette tip should rest gently on the bottom of the well, but not in the channel.



7. Typically the full 200 µL of sample cannot be recovered. 75–125 µL is the expected range.
8. Add sufficient Cells Sample Loading Buffer to the sample to bring the total volume to 210 µL.
9. Store recovered samples at -20°C until they are ready to be re-run. Ensure samples are at room temperature and well mixed before loading on a new chip. When re-running the sample, always use the same protocol to the original run.