



jnmedsys

Clarity™ Digital PCR System
User Manual

Version : 2016/01

For Research Use Only. Not for use in diagnostic procedures

The information in this guide is subject to change without notice.

Limited Warranty




JN Medsys provides a warranty period of 12 months for its Clarity™ Digital PCR System (from date of shipment unless otherwise noted in product manual) against any defects that are due to faulty materials or workmanship. Once JN Medsys is notified of such defects, it will, at its options, repair or replace the products that are proven to be defective. Replacement products may be new or equivalent in performance to new. Any product repaired or replaced under this warranty will be warranted for the remainder of the original product warranty period.



The warranty does not apply to defects resulting from:

1. Damage caused by misuse, neglect, accident or improper application or installation.
2. Improper or inadequate maintenance, repair or calibration not authorized by JN Medsys.
3. Software, interfacing, part or supplies not supplied by JN Medsys.
4. Operation not in accordance with the instructions furnished by JN Medsys.

Symbols

The following symbols are used in this document, and may be encountered when using the System.

Symbol	Description
	Caution
	Serial Number
	Consult Instructions

Symbol	Description
	Manufacturer
	Date of Manufacture

Warnings and Precautions

Please read the entire instructions for use before starting.

To facilitate the safe and proper use, and for safe and efficient operator maintenance of the System, please heed the following warnings and precautions as listed below:

- Do not operate the System until you have read the entire User Manual and have undergone training with a certified representative.

- Do not relocate the System after it has been installed, as it may result in loss of critical functional properties and void your warranty. Should you need to move your System, please contact JN Medsys or your designated representative.
- If in doubt, please contact the company for technical support at techsupport@jnmedsys.com

Using this product in a manner not specified in the User manual may result in protection impairment.

Regulation Compliance

The Clarity™ Digital PCR System complies with IEC/EN 61010-1, EN 61326-1. Prior to the operation of the system, an evaluation of the electromagnetic environment should always be conducted.

For more information, visit www.jnmedsys.com

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1. System Overview

Components of Clarity™ Digital PCR System

System Specifications

I. Specification Overview

II. System Hardware

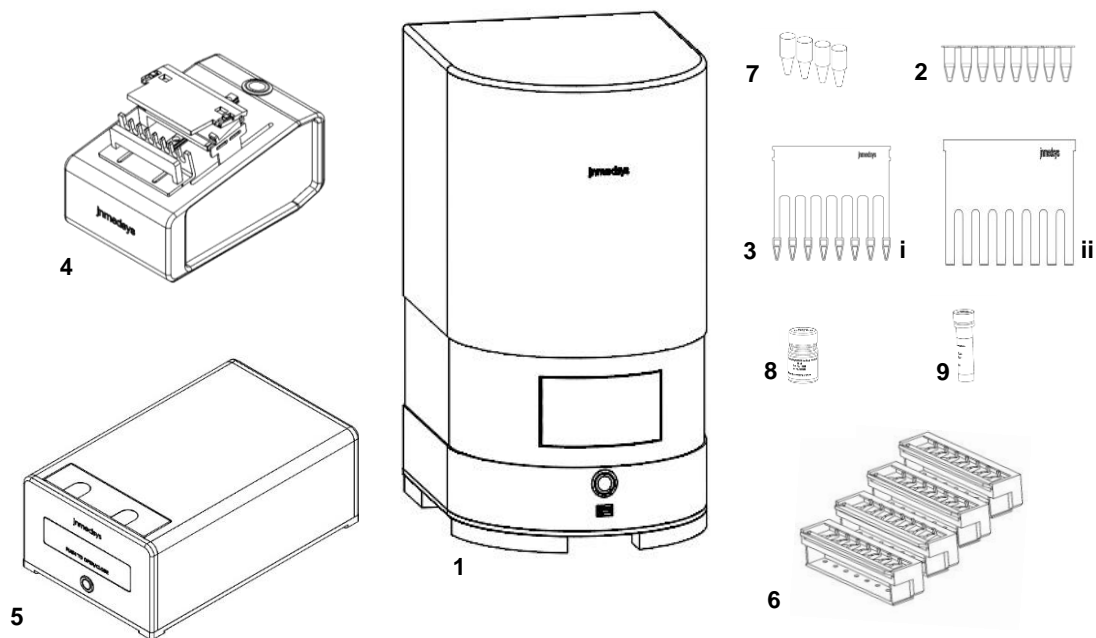
III. System Software

System Installation

General Workflow

Components of Clarity™ Digital PCR System

Clarity™ digital PCR system comprises of the following:



- | | |
|--|------------------------------------|
| 1. Clarity™ Reader | 5. Clarity™ Sealing Enhancer |
| 2. Clarity™ Tube-strip | 6. Viewing Jig for Clarity™ Reader |
| 3. Clarity™ Sample Loading Kit -
consists of a (i) slider and (ii) platform | 7. Thermal Cycler Spacers |
| 4. Clarity™ Auto Loader | 8. Clarity™ Sealing Fluid |
| | 9. Clarity™ JN Solution |

Additional general equipments/consumables required for performing digital PCR on Clarity™ system but not included in this package.

- Thermal cycler for standard 0.2 ml tubes, with adjustable ramp rate and adjustable height heated lid
- Microcentrifuge
- Vortexer
- Pipettes (P10 – P1000) and tips (10 – 1000 µL)
- DNase/RNase-free reaction tubes
- Microtube racks
- Lint-free wipes
- PCR grade water
- DNA decontamination solution
- Personal Protective Equipment (PPE)



Caution!

-
- *Always wear appropriate PPE (gloves and labcoats) to ensure safe and healthy working conditions. Discard all used consumables into biohazard waste bags..*
-

System Specifications

I. Specification Overview

System's Enclosures	Plastic casings			
System's Operating Temperature	0 to 40 °C			
System's Storage Temperature	-10 to 50 °C; 35% to 85 % relative humidity			
Power Requirements	Power Supply Units (PSUs) with power cord provided:			
	Instrument	Power adaptor	Input	Output
	Clarity™ Auto Loader	AFM45US12	100~240Vac 50/60Hz	12Vdc 4A
	Clarity™ Sealer Enhancer	ALM65US12	100~240Vac 50/60Hz	12Vdc 5.4A
Clarity™ Reader	AFM45US12	100~240Vac 50/60Hz	12Vdc 4A	
Pollution Degree	2			
Altitude	2,000m			
Reaction Volume	15 µl per tube			
System's Storage Temperature	0 to 70 °C; 95% Relative humidity, Non-condensing			
Partitions per Reaction	10 000			
Partition Volume	1.5 nl			
Reactions per Tube-strip	8			
Tube Capacity	0.2 ml			
Thermal Cycling	Any certified [^] thermal cycler equipped with adjustable ramp rate and heating block configured for 0.2 ml tubes			
Reader Throughput	32			
Detection Channels	2-colours : FAM and HEX/VIC			
Light Source	LED			
Detection Mode	End point			
Reader Interface	USB and Ethernet			
Dimensions, weight	<p style="text-align: center;"><u>Auto Loader</u> (W) 12 cm X (H) 9 cm X (D) 18 cm, 1 kg</p> <p style="text-align: center;"><u>Sealing Enhancer</u> (W) 18 cm X (H) 11 cm X (D) 29 cm, 4 kg</p> <p style="text-align: center;"><u>Reader</u> (W) 25 cm x (H) 42.5 cm x (D) 31 cm, 9.8 kg</p>			

*System refers to 3 instruments of Clarity Digital PCR system namely auto loader, sealing enhancer and reader

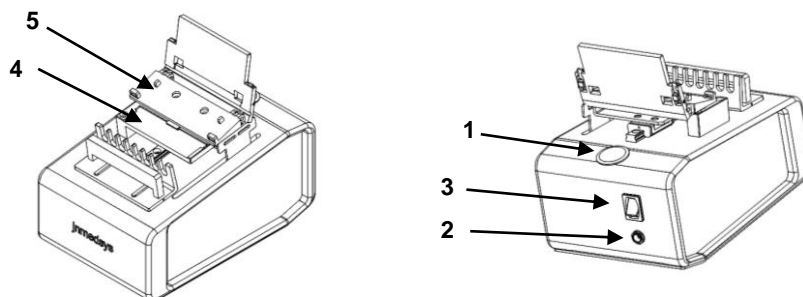
[^]Certified refers to IEC 61010-1, IEC 61010-2-010 and with applicable particular standards

II. System Hardware

Clarity™ Digital PCR System hardware consists of

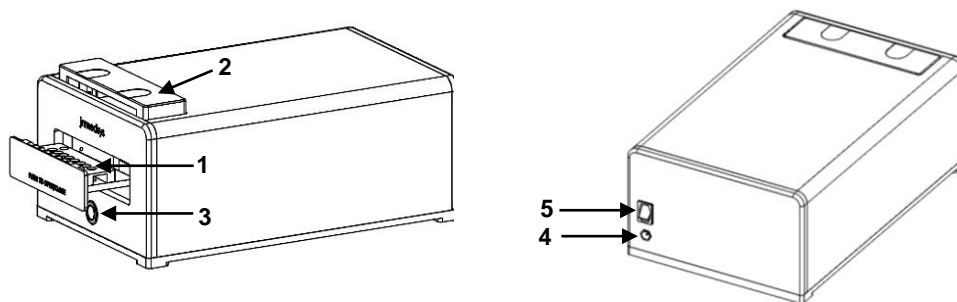
1. Clarity™ Auto Loader: To load sample onto tube-strip
2. Clarity™ Sealing Enhancer: To stabilize sample for sealing in tube-strip
3. Clarity™ Reader: To take and store data for offline analysis

1. Clarity™ Auto Loader



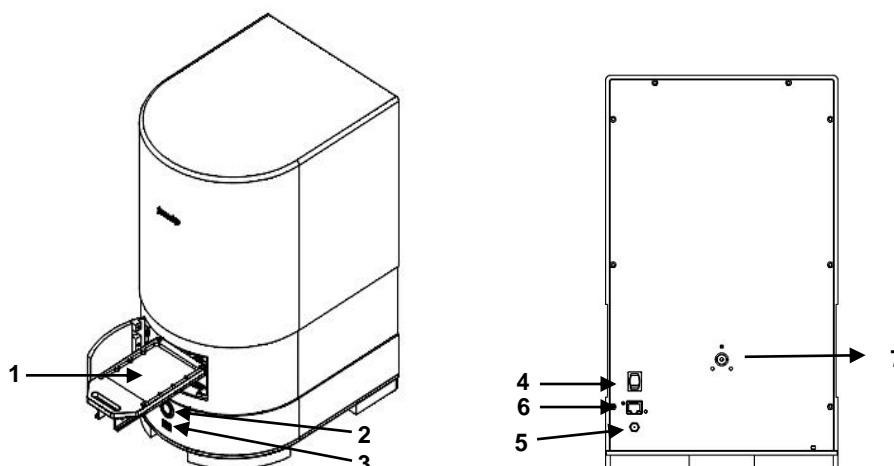
No.	Component	Description
1	Start button	Activates slider for sample partitioning
2	Power port	A 12V port that provides power to the instrument
3	Power switch	Switches the instrument on or off
4	Platform holder	Holds platform in position for sample partitioning
5	Slider holder	Holds slider in position for sample partitioning

2. Clarity™ Sealing Enhancer



No.	Component	Description
1	Side panel with tube-strip holder	Holds tube-strip during sample sealing
2	Top panel	Secures tube-strip in place for sealing
3	Start button	Initiates the sample sealing process
4	Power port	A 12V port that provides power to the instrument
5	Power switch	Switches the instrument on or off

3. Clarity™ Reader



No.	Component	Description
1	Tube-strip tray	Holds viewing jigs with tube-strips during data acquisition
2	Start button	For initiating different tasks of the reader
3	USB port	For downloading data from reader using a USB drive
4	Power switch	Switches the instrument on or off
5	Power port	A 12V port that provides power to the instrument
6	Ethernet port	For connection to computer via lan cable
7	Transportation screw	For securing reader during transportation



Note

- Remove transportation screw before connecting it to a power source.

III. System Software

The Clarity™ Digital PCR System comes with the Clarity™ Software for analysing digital PCR data. The software comprises proprietary and multi-level thresholding algorithms with standard poisson distribution statistics.

The software is compatible with 32-bit/64-bit Microsoft Windows 7 or later, and installation requires at least 4GB RAM and 25MB of free disk space.

System Installation

Electrical and Lab Space Requirement

- The reader, auto loader and sealing enhancer are provided with PSU and power cord.
- Each instrument is provided with a PSU as follows:
 - PSU model no: AFM45U12 (AC powered: 100 to 240 VAC, 50/60 Hz to 12 VDC, 4 Amp) are used for reader and auto loader.
 - PSU model no: ALM65US12 (AC powered: 100 to 240 VAC, 50/60 Hz for 12 VDC, 5.4 Amp) is used for sealing enhancer.
 - The System comes with a region-specific power cord.
- Refer to Section I (Specification Overview) on the dimension and weight of each individual instrument.
- Allocate a bench space of 40 cm (depth) x 65 cm working space (width) x 45 cm (height) for the System (not inclusive of a laptop), with an uninterrupted power source for 3 instruments within 1 meter. Additional space to be included for a laptop which is used to connect to the reader.
- Ensure that the System is not placed or used on a bench with instruments that emit significant vibrations, such as a centrifuge, as this may interfere with the System's performance especially the reader's functions.
- Fuses are included in the PSU provided with each instrument respectively. If damage, replace the PSU as specified for each of the instrument.

Instrument Installation Guide

- Connect instruments to power source using the PSU and power cords provided.
- Switch on the power of the instruments. When the start button turns solid blue, it indicates the instrument is ready for use.

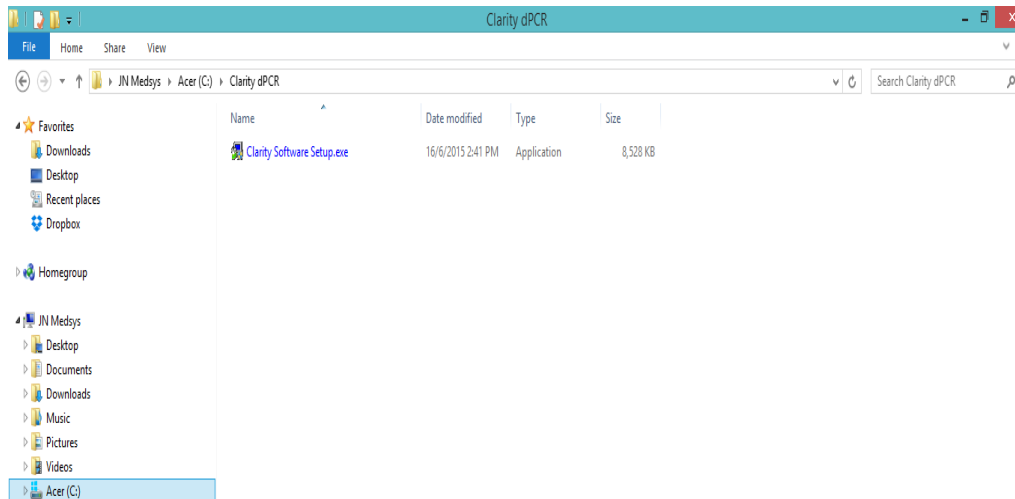


Caution!

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- *Always use dry gloves when handling power port, electrical switch and socket.*
 - *Be familiar with the operation of all instruments prior to use.*
 - *Only use the PSU supplied with the equipment from JN Medsys*
-

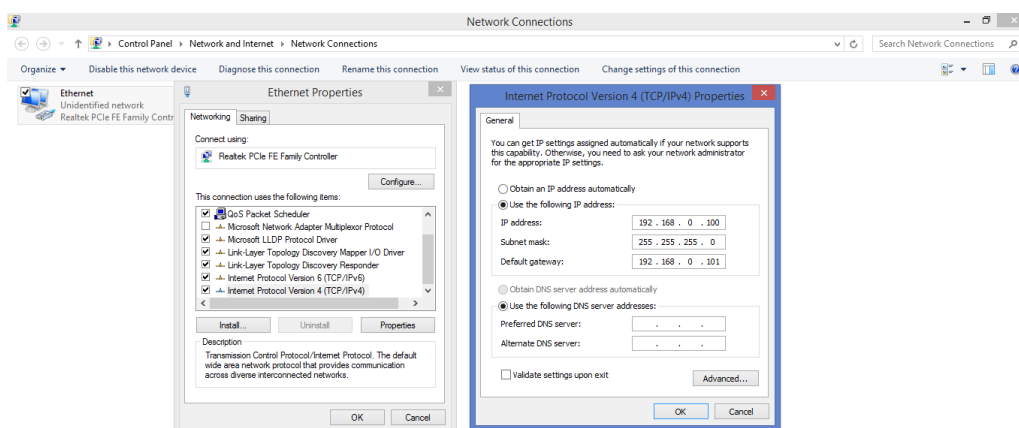
Software Installation Guide

- Double click on the “Clarity Software Setup” file to begin installation.



- After installation has completed, the software is ready for use.
- To connect the software to the reader, user need to assign an IP address to the computer.

Go to Control Panel\Network and Internet\Network Connections, right click on “Ethernet” and double click on “Internet Protocol Version 4 (TCP/IPv4)” to set the IP address as shown below. (IP address: 192.168.0.100; Subnet mask: 255.255.255.0)

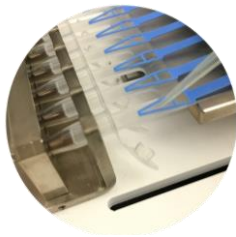


General Workflow



1. Reaction Mix Preparation

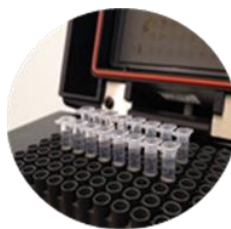
Prepare reaction mix by combining nucleic acid sample, primers, probes and PCR mastermix with Clarity™ JN Solution. EvaGreen® dye may be used as an alternative to hydrolysis probes when required.



2. Sample Partitioning

Load reaction mix into tube-strips using Clarity™ Auto Loader. The tube-strip design enables eight reaction mixtures to be partitioned simultaneously.

Seal tube-strips with Clarity™ Sealing Enhancer and Sealing Fluid after sample loading.



3. PCR Amplification

Perform PCR using a compatible thermal cycler.



4. Data Acquisition and Analysis

After PCR, load tube-strips into the Clarity™ Reader for data acquisition. Analyze data using the Clarity™ Software which determines the copy number present in the sample using Poisson statistics.

2. Reaction Mix Preparation

Materials Required

Designing the Digital PCR Assay

Preparation of DNA Samples

Preparation of Digital PCR Reaction Mix

Materials Required

The following materials are required to prepare digital PCR mix for analysis using the Clarity™ Digital PCR System.

- Clarity™ JN Solution (Catalog No. 12006)
- Fluorescent-labeled quantitative PCR reagents (For probe-based or DNA binding dye-based assays)
- Genomic or complementary DNA sample(s)

Note

- Always wear clean, powder-free gloves.
 - Clean lab benches and equipment periodically with DNA decontamination solution.
 - Prepare digital PCR reaction mixture in a clean and DNA-free environment.
-

Designing the Digital PCR Assay

The Clarity™ Digital PCR System is compatible with both probe-based (singleplex or duplex) and DNA binding dye-based (SYBR® Green or EvaGreen® dye) assays.

Below are some useful guidelines on designing a digital PCR assay for use on Clarity™ system:

- Amplicons should ideally be 100-300 bp in length. For SYBR Green®/ EvaGreen® assays, longer amplicon length (>150 bp) is recommended.
- Primers/probes should preferably be about 15-30 bp in length.
- GC content of primers/probes should be kept within 35–65%.
- Avoid sequence complementarity within a primer and between all primers used in the assay.
- Melting temperature (T_m) of primers is recommended to be around 60–64°C. Primer pairs should preferably have T_m values that are within 5°C.
- T_m of probes should be 6-8°C higher than that of the primers.

- For probe-based assays, double-quenched probes are recommended for obtaining optimal digital PCR results.

Preparation of DNA Samples

The quality of DNA sample is a crucial factor that can influence the results of Clarity™ digital PCR assays. We recommend that the target DNA template is extracted from the raw material using an optimized protocol. The $A_{260/280}$ and $A_{260/230}$ values of the DNA preparation should ideally be ~1.8 and ~2.0-2.2, respectively. While digital PCR assays are less susceptible to PCR inhibitors, it is advisable to remove as many inhibitors as possible during the DNA extraction step.

For digital PCR experiments performed on the Clarity™ system, the concentration of target DNA has to fall within the dynamic range of the system (**0 to 33600** copies/ 15 µl of reaction mix). In the event that the concentration of DNA sample exceeds the dynamic range, dilution of DNA sample is required prior to performing the digital PCR experiment.

The following guidelines may be used to assess whether dilution of a DNA sample is required:

1. Determine the genome size (in bp) which the target DNA originates from. Genome sizes of different organisms can be retrieved from the following database: <http://www.cbs.dtu.dk/databases/DOGS/index.html>.
2. Measure the concentration (in ng/µl) of DNA sample.
3. With the genome size and measured concentration of DNA sample, use the following formula to determine the copy number/µl of the DNA sample: $Y = \frac{[(\text{measured concentration}) * 6.022 * 10^{23}]}{[(\text{size}) * 10^9 * 650]}$
4. Based on the estimated copy number/µl of DNA sample, decide on the appropriate volume of DNA sample to be added into each digital PCR mixture. The recommended range of DNA concentration is 0-33600 copies/ 15 µl of reaction mix. Dilute DNA sample if it exceeds the dynamic range. Below are some examples on dilution of different DNA samples:

Sample	Target gene (Origin)	Genome Size (bp)	Measured concentration of DNA sample (ng/μl)	Estimated copy number/μl of DNA sample	Recommendation
1	RNaseP* (Human)	3.2 X 10 ⁹	19.3	11200	No dilution is necessary
2	RNaseP* (Human)	3.2 X 10 ⁹	10	5790	No dilution is necessary
3	GapA# (<i>Escherichia coli</i>)	4.6 X 10 ⁶	10	2014047	At least 60X dilution is required
4	EBNA# (Epstein-Barr virus)	1.7 X 10 ⁵	10	54497738	At least 1700X dilution is required

* Single copy gene per haploid genome (i.e. 2 copies per human cell)

Single copy gene in bacterial and viral genome, respectively

If the sample concentration or copy number per genome is unknown, it is recommended to carry out digital PCR assays using a dilution series of the sample to determine the optimal dilution range.

Preparation of Digital PCR Reactions

1. In a sterile microtube, prepare the digital PCR reaction mixture as follows for partitioning onto one chip. It is advisable to prepare excess reaction mix to account for potential pipetting error. Scale the volume accordingly, depending on the number of reactions required for each sample.

Component	Final Concentration/ Volume
PCR Mastermix	1X
Primers	100-300 nM*
Probes #	100-300 nM
Clarity™ JN Solution (20X)	1X
Template DNA	Refer to section on Preparation of DNA samples
PCR grade water	Top up to 15 μl

Omit probes from the reaction mixture when using mastermixes for SYBR Green® or EvaGreen® dye-based detection.

* For SYBR® Green or EvaGreen® dye-based assay, it is recommended to use 150 nM or lower concentration for optimal results.

2. Mix and briefly centrifuge the reaction mixture. Proceed to load mixture onto Clarity™ tube-strip.

3. Sample Partitioning

Materials Required

Sample Loading

Sample Sealing

Materials Required

The following are required for

Sample loading:

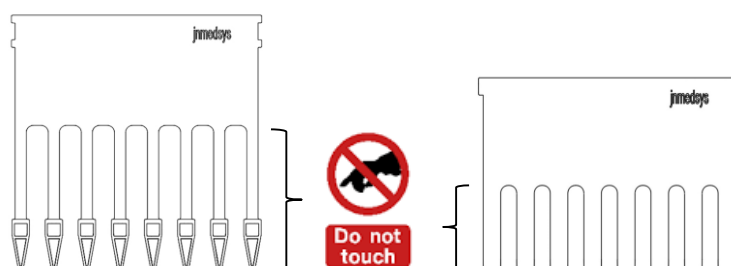
- Clarity™ Tube-strip (Catalog No. 12007)
- Clarity™ Auto Loader (Catalog No. 11002)
- Clarity™ Sample Loading Kit - consists of a slider and a platform (Catalog No. 12008)

Sample sealing:

- Clarity™ Sealing Enhancer (Catalog No. 11003)
- Clarity™ Sealing Fluid (Catalog No. 12005)

The following section provides important guidelines on sample loading and sealing. Follow the guidelines accordingly to ensure optimal partitioning of samples.

- The sample loading kit (with a slider and a platform) and tube-strip are individually packaged. Remove them from packaging just before sample loading to avoid contamination. Discard kit if packaging is broken or torn.
- Always handle tube-strip, slider and platform with powder-free gloves.
- Handle tube-strip with care. Do not apply pressure on it.
- The 8 “fingers” of the slider and platform come into contact with reaction mixture during sample loading. Avoid touching the “fingers” to prevent contamination of samples.



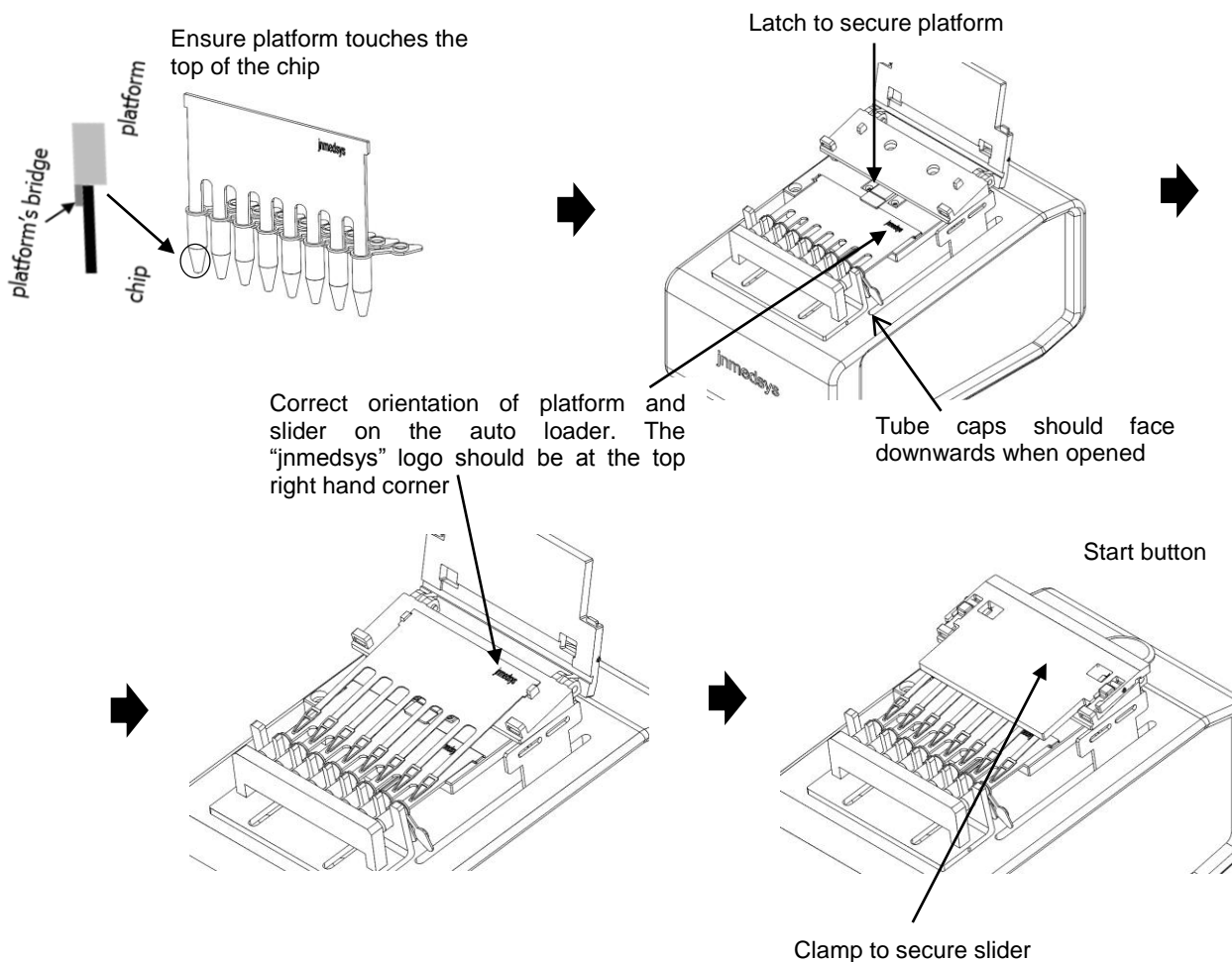
- For accurate sample tracking, use a permanent marker to label the caps of tube-strips. Do not label at the side of the tubes as it will interfere with optimal data acquisition.
- Carry out sample sealing immediately after loading of tube-strip.

- Surfaces of the instrument may require general cleaning. Use 70% ethanol for general wipe down with a slightly dampened cloth.

Sample Loading

Follow the steps below for sample loading:

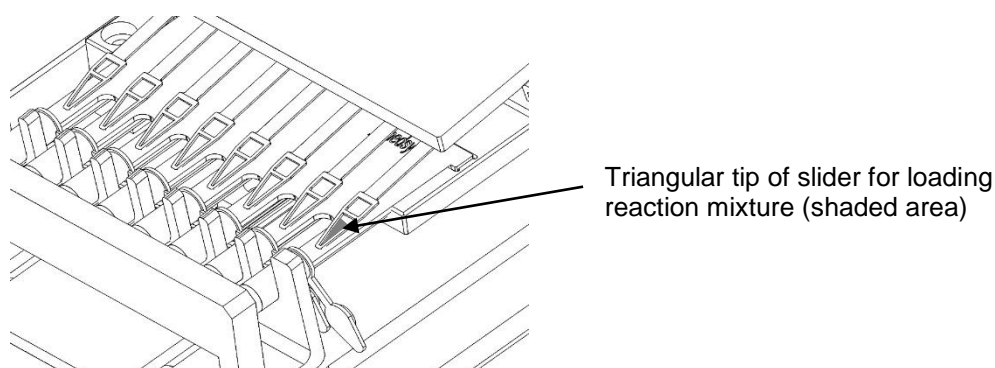
1. Switch on the Clarity™ Auto Loader.
2. Remove the sample loading kit and tube-strip from their packagings.
3. Place tube-strip on a PCR tube rack and open the tube caps.
4. Insert the platform into the tube-strip until it just touches the top of the chips (see figure below).
5. Place tube-strip with the inserted platform at their designated positions on the auto loader as shown below. Secure the platform with the latch.
6. Place the slider over the platform as shown in figure below. Secure the slider with the latch.



 **Note**

- Open and close tube caps only when tube-strips are placed on a PCR tube rack. This is to reduce risk of damaging the chips due to improper handling of tube-strips.
- Open tube caps only when required. Do not leave opened tube-strips unattended to avoid contamination.
- Ensure that the tube-strip, platform and slider are correctly assembled on the auto loader.
 - Tube caps should face downwards when opened.
 - “jnmedsys” logo should be found at the top right hand corner of slider and platform.

7. Pipette each of the 15 µl reaction mixture onto the triangular tip of slider as shown below.



8. After all eight reaction mixtures are loaded at the tips, press the start button to begin sample partitioning. The slider will move downwards and transfer the mixtures onto the respective chips. It will automatically retract and return to its original position after loading has completed.
9. Remove slider by releasing the latch. Discard the used slider.
10. Release the latch that secure the platform. Remove tube-strip together with the platform from the auto loader.
11. Remove platform from the tube-strip. Discard the used platform.
12. Transfer tube-strip to the Clarity™ Sealing Enhancer for sample sealing.

 **Note**

- Dispensing reaction mixture at the designated area is critical for proper sample loading.
- Handle the tube-strip with care. Do not shake or drop tube-strip to ensure optimal result.

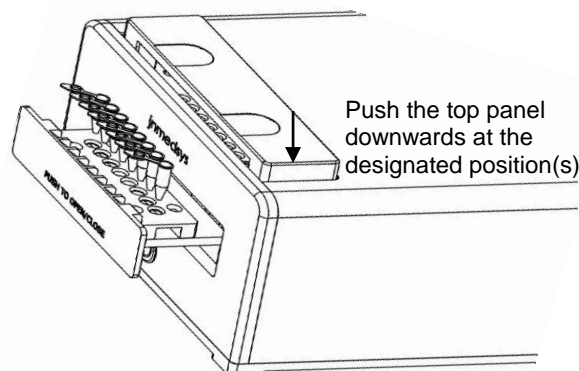
 **Caution!**

- Be familiar with the operation of auto loader and sample partitioning procedure before proceeding with experiment.

Sample Sealing

Follow the steps below for sample sealing:

1. Switch on the Clarity™ Sealing Enhancer.
2. Eject side panel and slot in tube-strip into the holder as shown below.



3. Close the side panel and push down the top panel.
4. Press the start button to begin sample sealing.
5. After sealing is completed, release the top panel and eject the side panel. Transfer tube-strip onto a rack.

Note

- To ensure the top panel is properly engaged for optimal sample sealing, always press the top panel downwards at the designated position(s).
- For proper operation of the sealing enhancer, always close the side panel before engaging the top panel. Likewise, always release the top panel before ejecting the side panel.

Caution!

- Be familiar with the operation of sealing enhancer and sample sealing procedure before proceeding with experiment.
- During the operation of sealing enhancer, some noise will be generated. Avoid prolong exposure.

6. Carefully dispense 230 µl of Clarity™ sealing fluid into each tube. Avoid bubble formation.
7. Close the caps of the tube-strip. Wipe tube-strip with lint-free wipes in case of sealing fluid leakage.
8. Transfer tube-strip to a thermal cycler.

Note

- Before using the sealing oil, mix well by shaking.
- For optimal results, start thermal cycling within an hour after sealing the tube-strip.
- Keep sealed tube-strips protected from light until you are ready to perform thermal cycling.

4. Thermal Cycling

Materials Required

Thermal Cycling

Materials Required

The following materials are required for thermal cycling

- Thermal cycler for standard 0.2 ml tubes, with adjustable ramp rate and adjustable
- Thermal cycler spacers (Catalog No. 13010)
- Loaded and sealed Clarity™ tube-strips

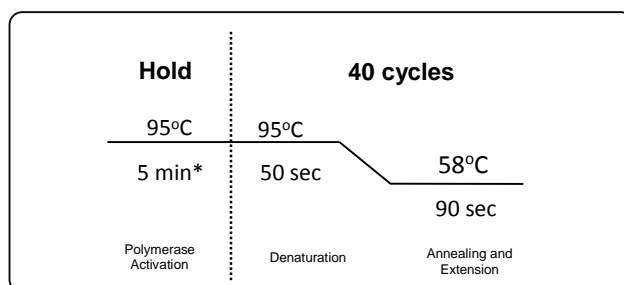
Thermal Cycling

General instructions for thermal cycling:

- Handle tube-strips with care. Do not apply pressure on the tube-strips to prevent leakage from chips.
- Thermal cycler spacers are to be loaded at the four corners of the thermal cycler heat block. This is to prevent deformation of the tube-strip during thermal cycling which will adversely affect data detection.

Follow the steps below for thermal cycling:

1. Perform thermal cycling using the recommended conditions below.



Ramp rate: 1°C/sec

*Time required for initial enzyme activation can be adjusted according to the requirement of polymerase used.

2. After thermal cycling, allow the thermal cycler heat block to equilibrate to room temperature before proceeding with data acquisition.



Note

- *Proceed to data acquisition immediately after the tube-strips have cooled to room temperature.*
-

5. Data Acquisition and Analysis

Materials Required

Data Acquisition

Data Analysis

Materials Required

The following materials are required for data acquisition and detection:

- Clarity™ Reader (Catalog No. 11004)
- Viewing Jigs for Clarity™ Reader (Catalog No. 13009)
- Lint-free wipes
- Water
- Clarity™ Software installed on a computer

Data Acquisition

General Instructions for Data Acquisition :

- The Clarity™ reader can acquire data from up to 4 tube-strips per run.
- Parameters for data acquisition can be modified using the Clarity™ software.
- Acquisition of data has to be performed immediately after thermal cycling when the tube-strips are cooled to room temperature.
- Handle tube-strips with care. Do not apply pressure on the tube-strips to prevent leakage from chips.
- Clean tube-strips with dry lint-free wipes to remove any stain/dust which will otherwise affect optimal data acquisition. Clean with water if necessary. Do not use ethanol or any other solvents.

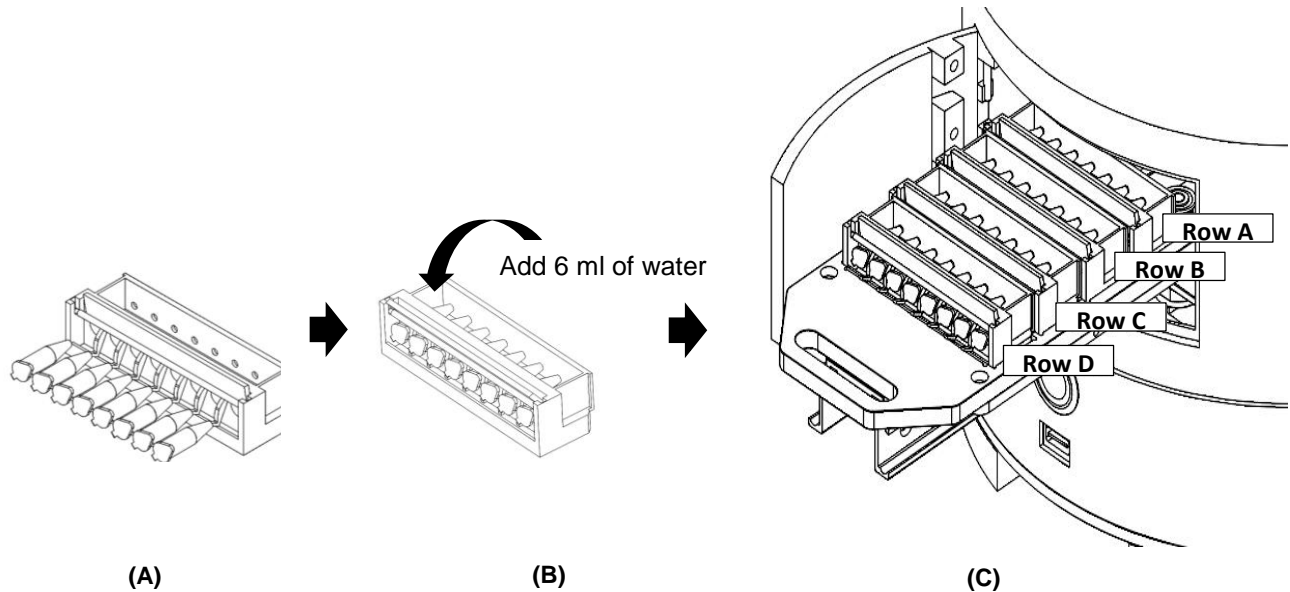


Caution!

-
- *Be familiar with the operation of reader and data acquisition procedure before proceeding with experiment.*
 - *When viewing jig is filled with water, handle it with care. Avoid spillage.*
-

Assembly of tube-strips into viewing jigs for data acquisition

1. After thermal cycling, insert each tube-strip into a viewing jig according to the figure below (A). Ensure that the tube-strip caps are facing upwards.




2. Fill each viewing jig with 6 ml of water (B).
3. Eject the reader tray using either the “Reader Control Mode” or “Load and Analyze Mode” (See below for details).
4. Lay each viewing jig horizontally on reader tray (C). Up to 4 viewing jigs can be fitted on the tray at one time. The row furthest from the tray door is designated as “Row A” while the row nearest to tray door is designated as “Row D”.
5. Push the tray until the reader automatically retracts it. Proceed with data acquisition.

Data acquisition can proceed via two approaches:

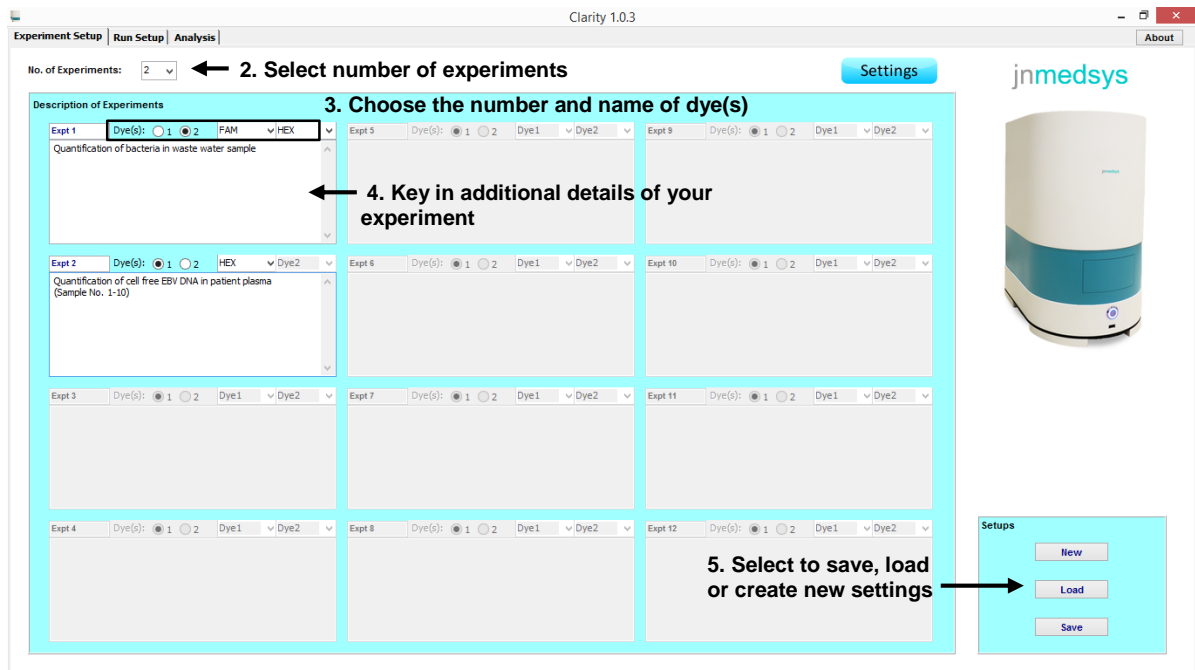
- (A) Reader Control Mode
- (B) Load and Analyze Mode

(A) Reader Control Mode

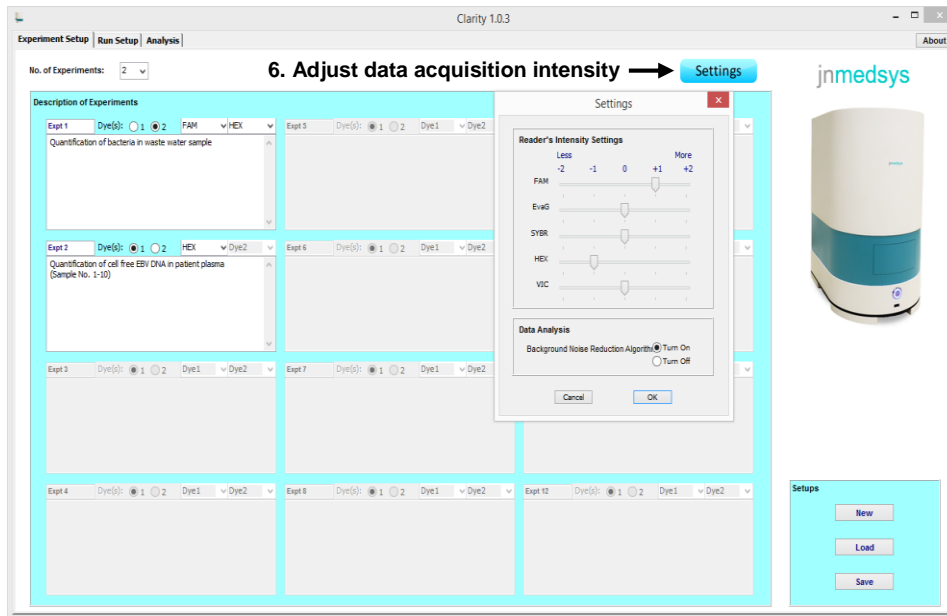
This mode of data acquisition requires the Clarity™ Reader to be connected to a computer pre-installed with Clarity™ Software. In this mode, the start button of the reader will not display any light and data acquisition can only be controlled through the software.

1. Switch on the Clarity™ Reader and open the Clarity™ Software  .

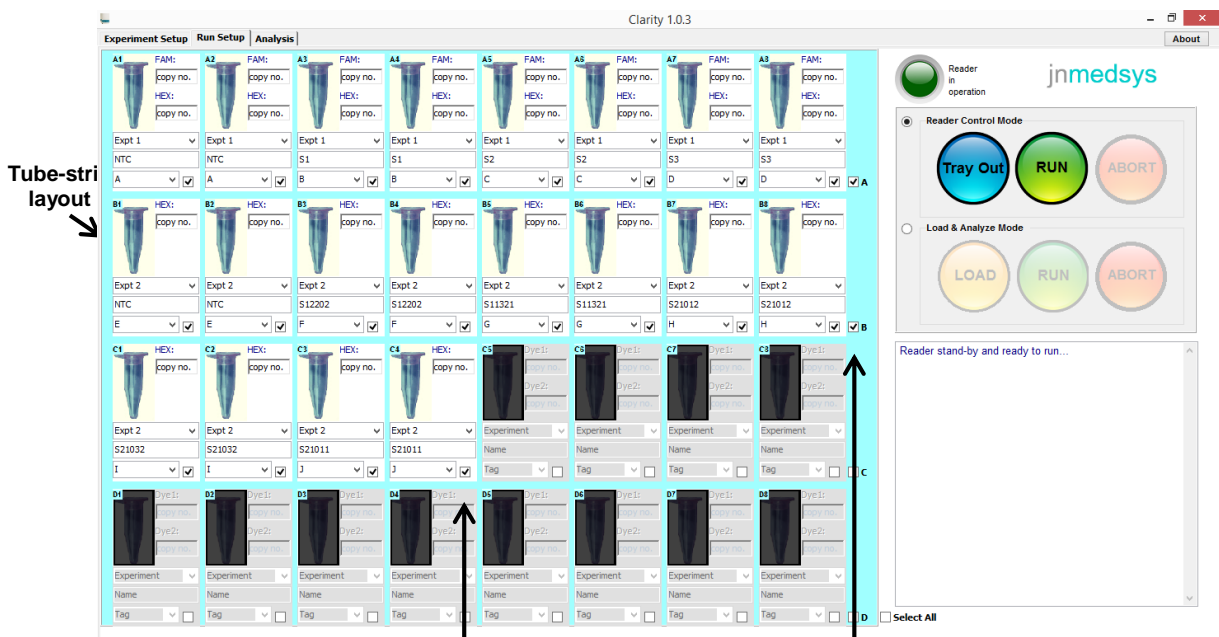
- Under the “**Experiment Setup**” tab, select the number of experiments to be analyzed. Up to 12 different experiments can be analyzed per run. Depending on the number of experiments chosen, the corresponding number of boxes will be enabled which allow entering of relevant experiment details.
- For each experiment, select the number and type of dye(s) used. An example is shown as below.
- Additional experimental details may also be entered in the white box as depicted.



- To save the experiment setup, click on the “Save” button located at “Setups” panel and select the destination folder to save the corresponding .jns file. To load the saved setup for subsequent experiments, click on the “Load” button and select the required .jna file. To initiate a new experimental setup, select the “New” button.
- Intensity for data acquisition may be customized for different assays. To adjust the intensity, click on the “**Settings**” button. In the pop-up box, slide the tab for the desired dye left or right to decrease or increase the data acquisition intensity. If settings are not adjusted, data acquisition will be performed at the default setting of 0. Click “OK” to continue.

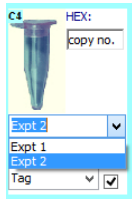


7. Proceed to the “Run Setup” tab which displays the tube-strip layout.
8. Select individual samples for data acquisition by checking the box next to “Tag”. To select the entire tube-strip, check on the boxes next to A/B/C/D. For a full analysis of four tube-strips, check on the box next to “Select All”. Once the boxes are checked, the corresponding blanks will be enabled for entering sample information.

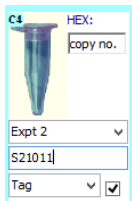


8. Check individual box to select the particular sample for data acquisition. Check “A/B/C/D” to select the entire row for data acquisition. Check “Select All” for a full acquisition of four tube-strips

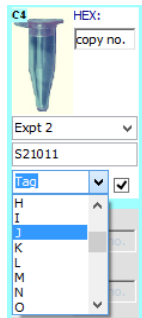
9. Assign the relevant experiment (**Expt**) to each sample to allow data acquisition to proceed according to the settings pre-defined under the “Experiment Setup” tab.



10. Enter sample name.



11. Group sample replicates by assigning them the same “**Tag**”. This is required for subsequent computation of mean DNA copies/ μ l, standard deviation and relative uncertainty (under Analysis Tab). Note: Completed information for this sample can be copied and pasted to the next replicate using the ctrl+c and ctrl+v commands.



12. After run setup is completed, click on the “Tray out” button to eject the reader tray.
13. Gently pull the tray outwards from its original position and load the viewing jigs fitted with tube-strips onto the tray (see above for instruction to assemble tube-strips into viewing jigs).
14. Gently push the tray back to its original position. The reader will then automatically retract the tray.
15. Click on the “**RUN**” button to begin data acquisition.

Note

- Ensure a vibration-free working table as movements will affect the quality of data acquisition.

(B) Reader Control Mode

This mode of data acquisition does not require the Clarity™ Reader to be connected to any computer (i.e. stand-alone mode). The reader is controlled using the start button and data acquisition is performed at the default setting of 0 .

1. Switch on the Clarity™ Reader. The start button should emit solid orange light. When the light turns blue, it indicates the reader is ready for use.

**Note**

-
- *Restart reader if the start button shows blinking orange light when switched on. If problem persists, contact JN Medsys for technical support.*
-

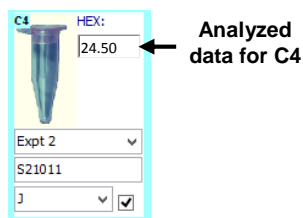
2. Press the start button once to eject the tray. The start button will emit blinking orange light after the tray is ejected.
3. Gently pull the tray outwards from its original position and load the viewing jigs fitted with tube-strips onto the tray (see above for instruction to assemble tube-strips into viewing jigs).
4. Gently push the tray back to its original position. The reader will then automatically retract the tray. Wait for the start button's light to switch from solid orange to solid blue.
5. To begin data acquisition, press and hold the start button until the blue light starts to blink. Blinking blue light indicates that the reader is in the process of data acquisition.
6. When data acquisition is completed, the start button will turn solid blue light.
7. A USB drive is required for downloading data from the reader. Follow the steps below to download the data:
 - a) Insert a USB drive into the port located below the start button. Wait until the start button's light turns from solid blue to blinking blue.
 - b) Press and hold the start button until blinking orange light appears. This indicates downloading of data is in progress.
 - c) When data acquisition is completed, the start button's light will switch to solid blue.
 - d) Remove thumbdrive from the reader and transfer the result folder (containing .bmp files) to a computer pre-installed with the Clarity™ Software.


 **Note**

- Ensure a vibration-free working table as movements will affect the quality of data acquisition.
- During data acquisition process (i.e. when start button shows blinking blue light), avoid pressing the start button unnecessarily as it will interfere with the data acquisition process.
- If required to stop the reader during the data acquisition process, press and hold the start button until it turns solid orange. The reader is ready to perform the next task when the start button switches to solid blue light.
- The reader is able to store data from up to five runs. However, it is advisable to download the data after each run to prevent override of data.

Data Analysis

- For the “**Reader Control Mode**”, data analysis is conducted simultaneously as data acquisition proceeds. The analyzed data for each sample will be displayed in the “Run Setup” tab as shown below. Once analysis for all samples is completed, the results will be transferred to the “Analysis” tab.



- For the “**Load and Analyze Mode**”, it is necessary to import the data (downloaded from the reader) into the Clarity™ software for analysis:
 - a) Open the Clarity™ Software .
 - b) Fill in the relevant details in the “Experimental Setup” tab (See “Data Acquisition using Reader Control Mode” for more details).
 - c) At the “Run Setup” tab, select “**Load and Analyzed Mode**”. Click on the “**LOAD**” button and select the required .bmp files (eg, BA1_*****.bmp, BA2_*****.bmp, etc) from the saved result folder.



- d) Assign the experiment, name and tag to each sample (See “Data Acquisition using Reader Control Mode” for more details).

e) Click “**RUN**” to begin analysis.

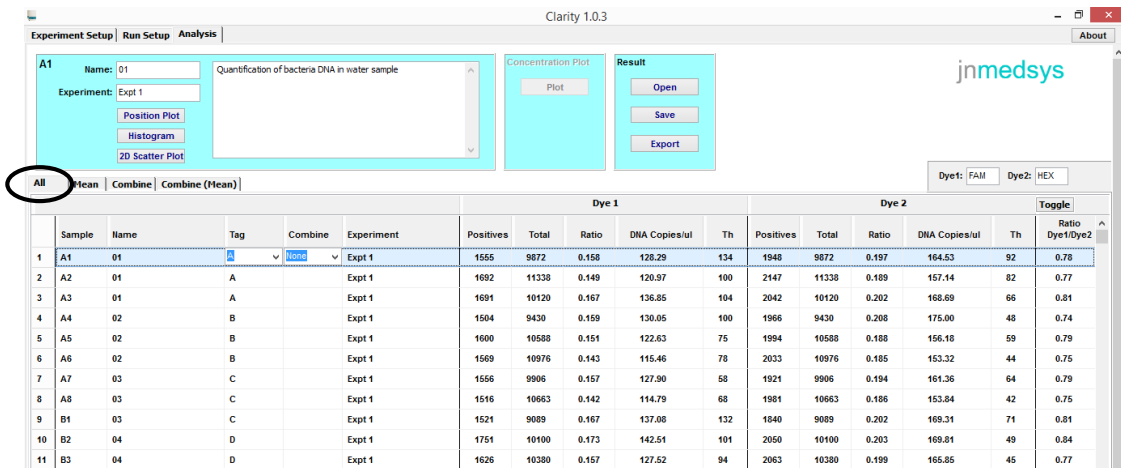


f) Once analysis for all samples is completed, the results will be transferred to the “**Analysis**” tab.

 **Note**

- The data files are named “**Bxy***” for **Dye 1** data and “**Gxy***” for **Dye 2** data. *x* corresponds to the row (A ,B,C or D) on the reader tray which the tube-strip was positioned for data acquisition. *y* corresponds to the position on each tube-strip (1-8).
To exemplify, BA1_***** and GA1_***** are dye 1 and dye 2 data from the first sample of the tube-strip positioned on row A of reader tray.
- When analyzing data from duplex-probe assays, it is not necessary to select the “Gxy” files as the software will automatically analyse them when the corresponding “Bxy” files are chosen.

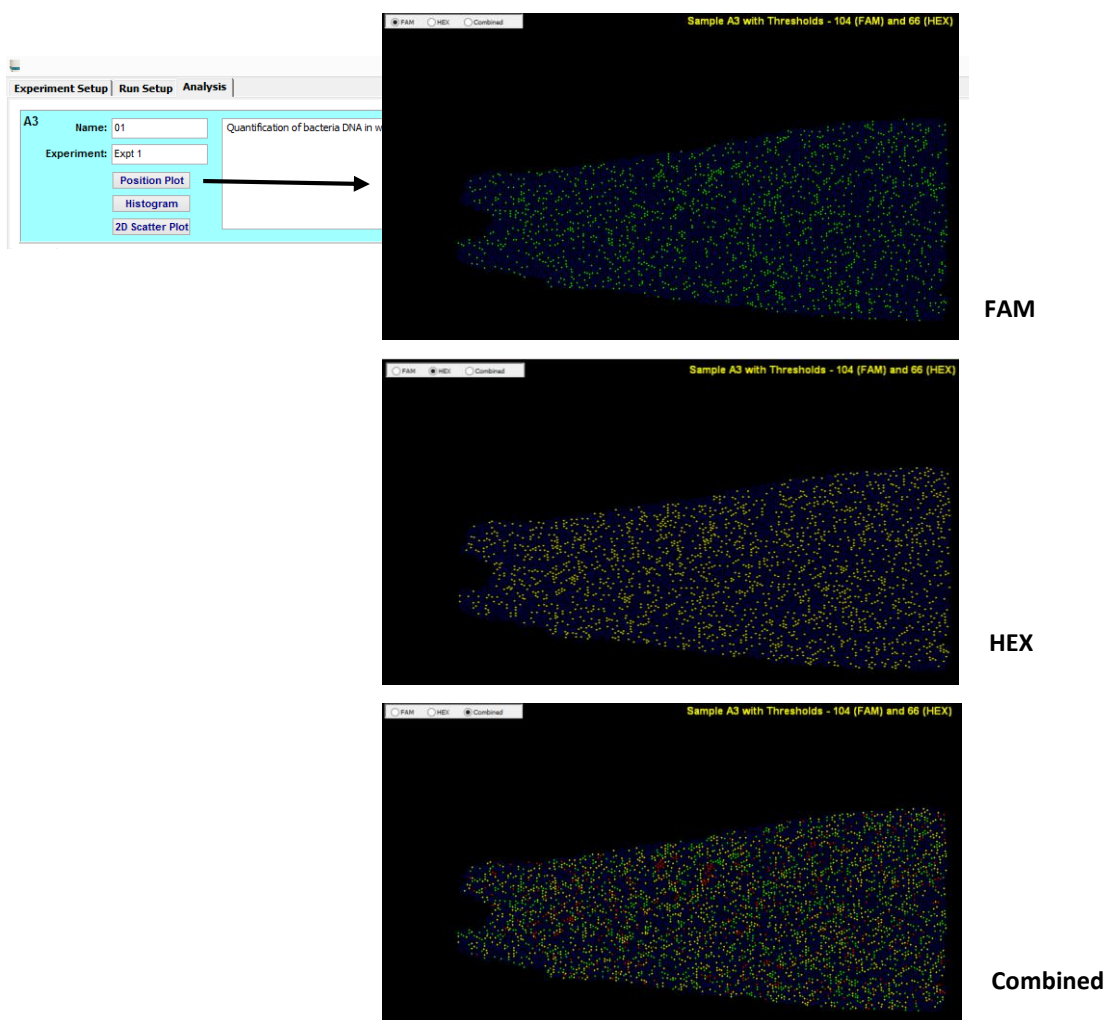
1. In the “**Analysis**” tab, select the “**All**” tab to view a summary of the number of **positive** partitions, **total** partitions, **ratio** of positive to total partitions, calculated **DNA copies/µl** and threshold (**Th**) for each sample.



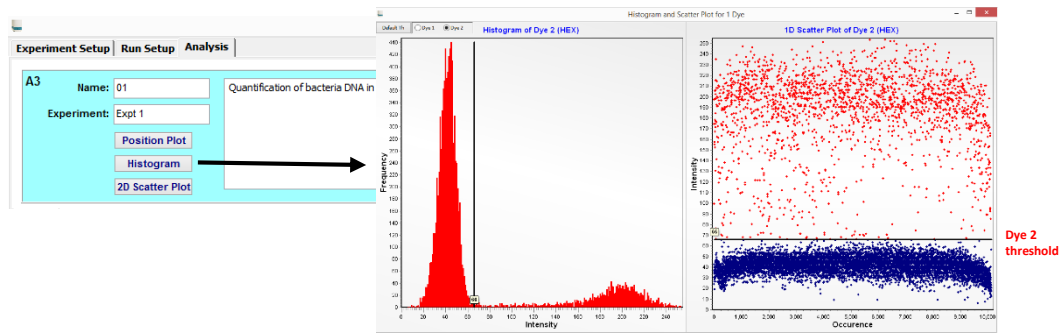
The screenshot shows the 'Analysis' tab in the Clarity 1.0.3 software. At the top, there are tabs for 'Experiment Setup', 'Run Setup', and 'Analysis'. Below these, there are several panels: 'A1' with a text area for 'Quantification of bacteria DNA in water sample', 'Concentration Plot' with a 'Plot' button, and 'Result' with 'Open', 'Save', and 'Export' buttons. A toolbar at the bottom left includes 'All', 'Mean', and 'Combine (Mean)' buttons, with 'All' circled in red. Below the toolbar is a large data table with columns for Sample, Name, Tag, Combine, Experiment, Positives, Total, Ratio, DNA Copies/ul, Th, and a 'Toggle' column for Dye 1 and Dye 2. The table contains 11 rows of data for samples A1 through B3.

Sample	Name	Tag	Combine	Experiment	Dye 1					Dye 2					
					Positives	Total	Ratio	DNA Copies/ul	Th	Positives	Total	Ratio	DNA Copies/ul	Th	Ratio Dye1/Dye2
1	A1	01		Expt 1	1555	9872	0.158	128.29	134	1948	9872	0.197	164.53	92	0.78
2	A2	01	A	Expt 1	1692	11338	0.149	120.97	100	2147	11338	0.189	157.14	82	0.77
3	A3	01	A	Expt 1	1691	10120	0.167	138.85	104	2042	10120	0.202	168.69	66	0.81
4	A4	02	B	Expt 1	1504	9430	0.159	130.05	100	1966	9430	0.208	175.00	48	0.74
5	A5	02	B	Expt 1	1600	10588	0.151	122.63	75	1994	10588	0.188	156.18	59	0.79
6	A6	02	B	Expt 1	1569	10976	0.143	115.46	78	2033	10976	0.185	153.32	44	0.75
7	A7	03	C	Expt 1	1556	9906	0.157	127.90	58	1921	9906	0.194	161.36	64	0.79
8	A8	03	C	Expt 1	1516	10663	0.142	114.79	68	1981	10663	0.186	153.84	42	0.75
9	B1	03	C	Expt 1	1521	9089	0.167	137.08	132	1840	9089	0.202	169.31	71	0.81
10	B2	04	D	Expt 1	1751	10100	0.173	142.51	101	2050	10100	0.203	168.81	49	0.84
11	B3	04	D	Expt 1	1626	10380	0.157	127.52	94	2063	10380	0.199	165.85	45	0.77

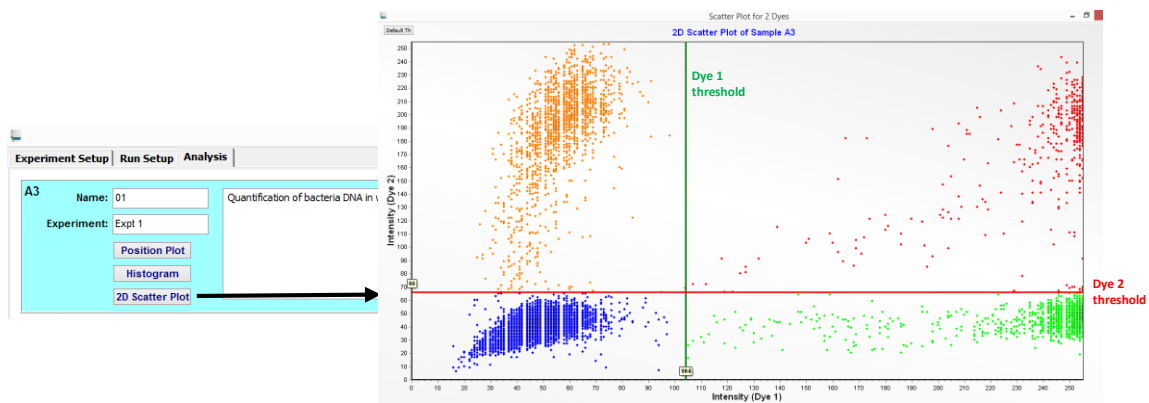
2. Click on the “**Position Plot**”, “**Histogram**” or “**2D Scatter Plot**” button to view the respective plots.
 - a) **Position Plot** shows the schematic distribution of positive and negative partitions in the sample. When using FAM, EvaGreen or SYBR® Green dyes, positive partitions are represented in green colour. When HEX or VIC dyes are used, positive partitions are coloured yellow. Partitions with both FAM and HEX/VIC signals are indicated in red. Below is an example of a sample analyzed using FAM and HEX dyes.



b) **Histogram** displays the histogram and 1D scatter plot of the results. In these plots, threshold adjustments can be made.



c) **2D Scatter Plot** shows the cluster intensities of dye 1 vs dye 2. Threshold adjustments can be done manually.

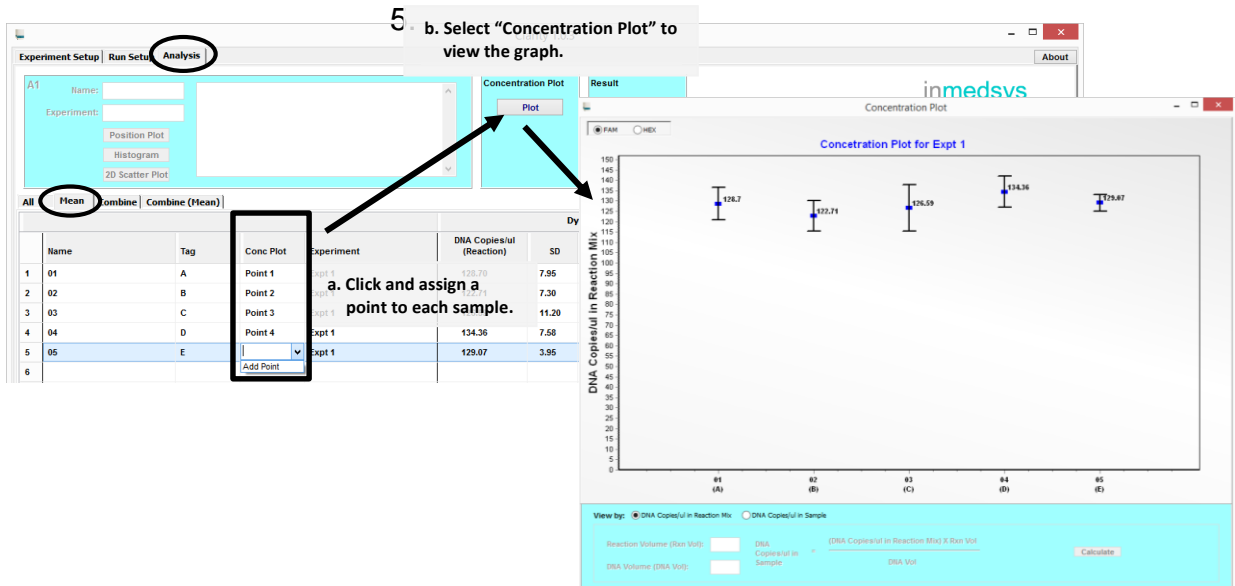


- Note :
- Green - Partitions with dye 1 signals only
 - Orange - Partitions with dye 2 signals only
 - Blue - Partitions with negative signals for both dyes
 - Red - Partitions with both dye 1 and dye 2 fluorescence signals

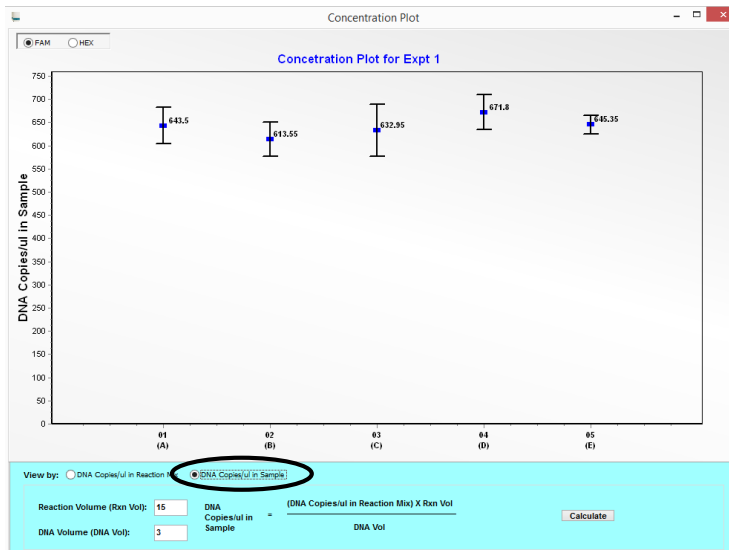
3. Select the **“Mean”** tab to view a summary of the mean DNA copies/μl, standard deviation (SD) and relative uncertainty (RU) of the replicates. Ensure that the replicates of the same sample are grouped under the same alphabet tag.

Name	Tag	Conc Plot	Experiment	Dye 1			Dye 2			Ratio Dye1/Dye2
				DNA Copies/ul (Reaction)	SD	RU	DNA Copies/ul (Sample)	DNA Copies/ul (Reaction)	SD	
1	01	A	Expt 1	128.70	7.95	0.0618	163.45	5.85	0.0358	0.79
2	02	B	Expt 1	122.71	7.30	0.0595	161.50	11.78	0.0729	0.76
3	03	C	Expt 1	126.59	11.20	0.0885	161.50	7.74	0.0479	0.78
4	04	D	Expt 1	134.36	7.58	0.0564	168.35	2.17	0.0129	0.80
5	05	E	Expt 1	129.07	3.95	0.0306	166.41	5.28	0.0310	0.78

- In the same tab, points can be assigned to each sample under the “**Conc Plot**” column for the generation of “**Concentration Plot**”. The graph will display the mean DNA concentration and SD of the sample replicates for each dye.



- By default, the concentration plot shows the “DNA copies/μl in **reaction mix**”. To determine the DNA concentration in the initial sample, select “DNA copies/μl in **sample**”. Enter the “Reaction Volume” and “DNA Volume” used accordingly (default is 15μl and 3μl, respectively) and click the “**Calculate**” button to generate the DNA concentration in the DNA sample.



6. For samples with low DNA concentration (e.g. ≤ 0.5 copies/ μ l, ~ 7 positives in 10,000 partitions), data from multiple replicates can be combined to generate a single data with higher number of total well count. These data should be assigned the same tag. An example is shown below:

(a) In the “All” tab, group the replicates into a single data under the “Combine” column.

Experiment Setup | Run Setup | **Analysis**

A6 Name: DNA with low count Experiment: Expt 1

Concentration Plot Plot

Result Open Save Export

All Mean Combine Combine (Mean)

					Dye 1						
Sample	Name	Tag	Combine	Experiment	Positives	Total	Ratio	DNA Copies/ul	Th	Positives	Tot
1	A1	DNA with low count	A	1	Expt 1	3	8252	0.000	0.27	53	
2	A2	DNA with low count	A	1	Expt 1	3	9027	0.000	0.26	66	
3	A3	DNA with low count	A	1	Expt 1	3	9027	0.000	0.25	85	
4	A4	DNA with low count	A	2	Expt 1	4	8806	0.000	0.34	44	
5	A5	DNA with low count	A	2	Expt 1	3	9307	0.000	0.24	50	
6	A6	DNA with low count	A	2	Expt 1	3	9621	0.000	0.23	156	

Under Combine, group data from multiple chips into a single data with higher number total wells

(b) Select the “Combine” tab to view the combined data.

Experiment Setup | Run Setup | **Analysis**

A1 Name: Experiment:

Concentration Plot Plot

Result Open Save Export

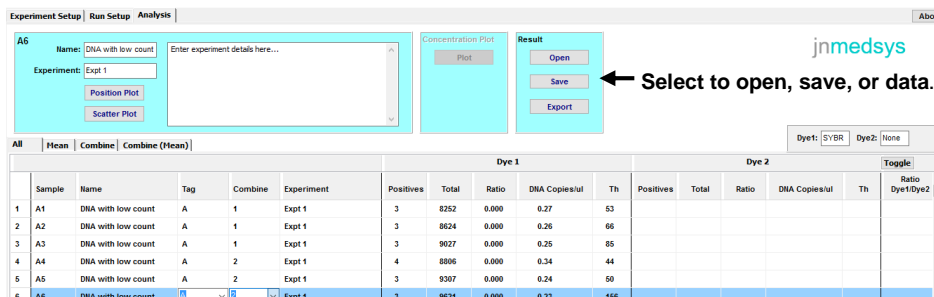
All Mean **Combine** Combine (Mean)

					Dye 1						
Name	Tag	Combine	Experiment	Positives	Total	Ratio	DNA Copies/ul	Positives	To		
1	DNA with low count	A	1	Expt 1	9	25903	0.000	0.26			
2	DNA with low count	A	2	Expt 1	10	27734	0.000	0.27			

Under All tab, display all individual data							Under Combine tab, display only the combined data			
Sample	Tag	Combine		Positives	Total	DNA Copies/ μ l	Positives	Total	DNA Copies/ μ l	
A1	A	1		3	8252	0.27	3+3+3 =9	8252+8624+9027 = 25903	0.26	
A2	A	1		3	8624	0.26				
A3	A	1		3	9027	0.25				
A4	A	2		4	8806	0.34	4+3+3 =10	8806+9307+9621 = 27734	0.27	
A5	A	2		3	9307	0.24				
A6	A	2		3	9621	0.23				

Example of how data from multiple replicates are combined to generate data in the “Combined” tab.

7. Select “**Combine (Mean)**” tab to view the mean concentration, SD and RU of the combined data. Concentration plot for the combined data can also be generated (See points 4 and 5 for details).
8. In the All, Mean, Combine and Combine (Mean) tabs, the last column displays the ratio of DNA copies/ μ l in dye 1 to that in dye 2. Click “**Toggle**” to change the ratio from (DNA copies/ μ l in dye 1 / DNA copies/ μ l in dye 2) to (DNA copies/ μ l in dye 2 / DNA copies/ μ l in dye 1).
9. Click “Save”, “Open” or “Export” to save, open a previously saved result file or export the results into CSV file format (***(All).csv, (***(Mean).csv, (***(Comb).csv, (***(CombMean).csv), respectively. CSV file can be used with any spreadsheet program such as Microsoft Excel, Open Office Calc. or Google Spreadsheets.



6. Troubleshooting

The following table summarizes some of the common issues that may be encountered during the use of Clarity™ digital PCR system.

Problems	Possible Cause	Recommended Action
Sample Loading and Sealing		
Excess reaction mix retained on chip surface after sample sealing.	More than 15 µl reaction mix was loaded.	Ensure that at most 15 µl of reaction mix was loaded.
	Reaction mix was not properly loaded.	Repeat sample sealing process.
Not able to open or close the side panel of the sealing enhancer.	The top panel is engaged.	Release the top panel.
Data Acquisition		
Start button of Clarity™ reader remains solid orange colour for more than 1 min.	The reader is not responding.	Restart the instrument.
Data acquisition is not completed after 20 min.		If the problem persists, contact JN Medsys for technical support.
Data Analysis		
Signals detected in non-template controls.	Contamination of assay reagents and/or contamination of samples during loading process.	Prepare reaction mix in a template-free environment. A uracil-DNA glycosylase step may be incorporated prior to PCR to remove product carryover from a previous reaction.
No signals detected for positive samples.	Inappropriate assay set-up.	Ensure that correct primers and probes were used and that DNA template has not degraded. Use a compatible PCR mastermix.
	Inappropriate brightness level used during data acquisition.	Repeat data acquisition using a higher brightness level and repeat data analysis.
Signals were detected in all wells (i.e. saturation).	Excess DNA template was used.	Repeat assay using a dilution series of the sample to determine the optimal dilution range.
	Too much primers/probes were used.	Repeat assay with lower concentrations of primers and/or probes.
	Inappropriate brightness level used during data acquisition.	Repeat data acquisition using a lower brightness level and repeat data analysis.

Need technical support? Contact our technical support at techsupport@jnmedsys.com.

7. Ordering Information

For more information on Clarity™ Digital PCR System, please visit our website at www.jnmedsys.com

To request for quotes or make an enquiry, email us at info@jnmedsys.com.

System, kit and consumables information

Catalog No.	Description
10001	Clarity™ Digital PCR System <ul style="list-style-type: none"> ▪ Clarity™ Tube-strip (96 reactions) ▪ Clarity™ Reader ▪ Clarity™ Auto Loader ▪ Clarity™ Sealing Enhancer ▪ Clarity™ Sample Loading Kit ▪ Clarity™ JN Solution (100 ul) ▪ Clarity™ Sealing Fluid (30 ml) ▪ Viewing Jig for Clarity™ Reader (4 pieces) ▪ Thermal Cycler Spacers (4 pieces)
11002	Clarity™ Auto Loader (Model no: C102)
11003	Clarity™ Sealing Enhancer(Model no:C103)
11004	Clarity™ Reader(Model no:C104)
10011	Clarity™ Consumables Package <ul style="list-style-type: none"> ▪ Clarity™ Tube-strip (96 reactions) ▪ Clarity™ Sample Loading Kit (96 reactions) ▪ Clarity™ Sealing Fluid (30 ml)
12007	Clarity™ Tube-strip (96 reactions)
12008	Clarity™ Sample Loading Kit (96 reactions)
12005	Clarity™ Sealing Fluid (30 ml)
12006	Clarity™ JN Solution (100 µl)
13009	Viewing Jig for Clarity™ Reader (4 pieces)
13010	Thermal Cycler Spacers (4 pieces)

8. Contact



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