SARS-CoV-2 Panel

INSTRUCTIONS FOR USE

Multiplex RT-PCR/MALDI-TOF test intended for the qualitative detection of nucleic acid from SARS-CoV-2

REF 13279, 13279F, 13278, 13278D, 13281, 13281D

IFU-CUS-001 R02
6/17/20

Note: Pending FDA review. The test has been validated, but FDA's independent review of this validation is pending.

IVD

For *in vitro* diagnostic use.
Pending FDA review

Agena® BIOSCIENCE

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See www.agenabio.com for Warranty and for Product Terms & Conditions. All Agena software, user guides, and other user documentation referred to in this document are available on the Agena customer support portal or from Agena Customer Support.

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[0819]

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[0818]

Revision History

Revision Number	Date	Changes Made
R02	6/17/20	Correction to panel name and FDA statement.
R01	6/14/20	Initial release.

REF	13279	Σ	960 tests
REF	13279F	Σ	960 tests
REF	13278	Σ	768 tests
REF	13278D	Σ	768 tests
REF	13281	Σ	3840 tests
REF	13281D	Σ	3840 tests

Contents

Chapter 1

SARS-CoV-2 Panel Product Information

1.1 Intended use

The SARS-CoV-2 Panel, for use on the MassARRAY® System, is a multiplex RT-PCR/ MALDI-TOF test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory samples (nasopharyngeal swab, oropharyngeal swab) and bronchoalveolar lavage (BAL) samples from individuals suspected of COVID-19 by their healthcare provider. The SARS-CoV-2 Panel is pending FDA review, and is for use in US laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory and bronchoalveolar lavage (BAL) samples during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The SARS-CoV-2 Panel is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of reverse transcriptase-PCR and *in vitro* diagnostic procedures. The SARS-CoV-2 Panel is pending FDA review.

1.2 Product description

The panel consists of 5 assays targeting the genome of SARS-CoV-2: three probe the viral nucleocapsid (*N*) gene and two probe *ORF1* and *ORF1ab*. The panel also contains an MS2 phage assay that monitors RNA extraction.

Table 1.1 SARS-CoV-2 Panel Panel Content

Assay Name	Region
SC2-N1	Nucleocapsid
SC2-N2	Nucleocapsid
SC2-N3	Nucleocapsid
SC2-ORF1	ORF1
SC2-ORF1ab	ORF1ab
MS2 Phage	-

1.3 Materials provided

The following items (Table 1.2) are provided in the SARS-CoV-2 Panel Kit. Upon receipt, store the items as described. The SARS-CoV-2 Panel comes in six different formats (Table 1.3).

Table 1.2 SARS-CoV-2 Panel Kit Contents

Materials Provided	Shipping Condition	Storage Temperature	Storage Location (see Table 3.1)
SARS-CoV-2 Panel • SARS-CoV-2 Panel PCR Primers • SARS-CoV-2 Panel Extend Primers • UNG (heat labile) • MMLV Enzyme • RNase Inhibitor • MS2 Control	Dry Ice	-25°C to - 10°C	Lab Area 2
PCR Reagent Set with dUTP • $MgCl_2$, 25 mM • 10X PCR Buffer • dUTP/dNTP Mix • PCR Enzyme	Dry Ice	-25°C to - 10°C	Lab Area 2
iPLEX® Pro Reagent Set • 3-Point Calibrant • iPLEX Termination Mix • iPLEX Buffer Plus, GPR • iPLEX Pro Enzyme • SAP Buffer • Shrimp Alkaline Phosphatase (SAP)	Dry Ice	-25°C to - 10°C	Lab Area 2
SpectroCHIP Arrays	Ambient Temperature	15°C to 25°C	Lab Area 3

Table 1.3 SARS-CoV-2 Panel Kits

Part #	Number of tests	Type of SpectroCHIP Array
REF 13279	Σ 960 tests	SpectroCHIP-96, 10 x 96
REF 13279F	Σ 960 tests	SpectroCHIP CPM-96, 10 x 96
REF 13278	Σ 768 tests	SpectroCHIP-384, 2 x 384
REF 13278D	Σ 768 tests	SpectroCHIP CPM-384, 2 x 384
REF 13281	Σ 3840 tests	SpectroCHIP-384, 10 x 384
REF 13281D	Σ 3840 tests	SpectroCHIP CPM-384, 10 x 384

1.4 Materials required but not provided

"MLS" indicates that the item is available from major laboratory suppliers.

Table 1.4 Materials Required but not Provided

Item	Source/Specification
Instruments and Equipment	
MassARRAY System with: • Typer software v5.0.1 or greater • RT-Workstation v4.1 or greater • Chip Prep Controller v2.2 or greater (if using Chip Prep Module)	Agena Bioscience • MassARRAY System with Chip Prep Module (CPM) 96 • MassARRAY System with Chip Prep Module (CPM) 384 • MassARRAY Analyzer 4 and Nanodispenser RS1000 96 • MassARRAY Analyzer 4 and Nanodispenser RS1000 384
Plate centrifuge	MLS; Max. RCF with plate rotor: 3,486 x g
Vortex	MLS; Variable speed, suitable for tubes and plates
Mini tube centrifuge	MLS; Recommend additional rotor option for 2 x 8-tube PCR strips
Refrigerated tube centrifuge	MLS; Capacity up to 12,000 ng
Thermocycler	MLS; With appropriate plate block; max ramp rate 4°C/second
PCR workstation with UV irradiation	MLS; UV light with timer; dual UV bulb preferred
Additional Software	
SC2 Report v1.0 or greater	Agena's customer support portal
RNA Extraction Materials	
TRI reagent	MLS; phenol and guanidine thiocyanate in a monophase solution
Chloroform	MLS; HPLC-grade
Glycoblue™ coprecipitant	ThermoFisher, #AM9515
Ethanol	MLS; Absolute (200 proof), molecular biology grade
Nuclease-free water	MLS; molecular biology grade
Isopropanol, molecular biology grade	Sigma Aldrich (#I9516) or equivalent
Additional Reagents	
Positive SARS-CoV-2 RNA Control	Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 (#102019)
HPLC-grade water	MLS; Residue after evaporation ≤2ppm; Sterile, nuclease- and DEPC-free; >18.2 MΩ
DNA AWAY™	MLS
Type 1 water or deionized water	MLS; NCCLS, CAP or ATSM; >18.2 MΩ
Clean Resin	Agena Bioscience, #08060 [40g], #08040 [28g]

Item	Source/Specification
Labware	
Electronic multichannel pipettes and filtered tips (Optional; can use manual multichannel pipettes)	MLS; 8- or 12-channel electronic adjustable tip spacing pipette; 0.5 µL -12.5 µL
Manual multichannel pipettes and filtered tips	MLS; 8- or 12-channel pipette 0.5 µL -20 µL 20 µL - 200 µL
Single channel pipettes and filtered tips	MLS; 0.1 µL - 2 µL 0.5 µL -10 µL 10 µL -100 µL 100 µL -1000 µL
Microtubes	MLS; RNase-, DNase-, human DNA-, and PCR inhibitor-free Volume: 1.5 mL, 5 mL
PCR strip tubes (optional)	MLS; 8- or 12-well strips with caps; volume: 0.2 mL
Tube racks	MLS
Sealing roller tool and paddle	MLS
Disposable pipetting reservoirs	MLS; DNase- and RNase-free; volume: 25 mL and 50 mL; sterile
Clear adhesive plate seals	MLS; Strong adhesive, -20°C to 120 °C
MassARRAY System with Chip Prep Module 96	
96-well semi-skirted, colored microtiter plates OR 96-well non-skirted microtiter plates	ThermoFisher MicroAmp® EnduraPlate™ #4483343 or equivalent.; Working vol.: 0.2 mL; Max well volume: 0.25 mL Thermo Scientific™ PCR Plate #AB0600L or equivalent; Working volume: 0.2 mL; Max fill volume: 0.3 mL
Isopropanol	MLS
MassARRAY System with Chip Prep Module 384	
384-well microtiter plates	Thermo Scientific™ 384-Well Full Skirted PCR Plate #TF-0384 or equivalent; Full skirted: Working volume: 25 µL, Max volume: 40 µL
Isopropanol	MLS
MassARRAY Analyzer 4 and Nanodispenser RS1000 System (96 format)	
96-well non-skirted microtiter plates	Thermo Scientific™ PCR Plate #AB0600L or equivalent; Working volume: 0.2 mL; Max fill volume: 0.3 mL
96-well fully skirted MTP plate base	Agena Bioscience #179108
Resin dimple plate and scraper	Supplied with Agena Bioscience MassARRAY System with RS1000 Nanodispenser
Plate/tube rotator	MLS; 360° rotation, with standard rotisserie
NaOH	MLS; 0.1 M
Ethanol	MLS; Absolute (200 proof); molecular biology grade

Item	Source/Specification
MassARRAY Analyzer 4 and Nanodispenser RS1000 System (384 format)	
384-well microtiter plates	Thermo Scientific™ 384-Well Full Skirted PCR Plate #TF-0384 or equivalent; Full skirted: Working volume: 25 µL, Max volume: 40 µL
Resin dimple plate and scraper	Supplied with Agena Bioscience MassARRAY System with RS1000 Nanodispenser
Plate/tube rotator	MLS; 360° rotation, with standard rotisserie
NaOH	MLS; 0.1 M
Ethanol	MLS; Absolute (200 proof); molecular biology grade

1.5 Workflow

After sample collection, RNA extraction is performed using a TRI-based method. RT-PCR with iPLEX® Pro chemistry then amplifies target regions of interest. After the inactivation of unincorporated dNTPs, a sequence-specific primer extension step is performed using the supplied Extend primers and iPLEX Pro reagents.

The extension products (analyte) are desalted, transferred to a SpectroCHIP® Array (a silicon chip with pre-spotted matrix crystal) and then loaded into the MassARRAY Analyzer (a MALDI-TOF mass spectrometer). The analyte/matrix co-crystals are irradiated by a laser, inducing their desorption and ionization. The positively charged molecules accelerate into a flight tube towards a detector. Separation occurs by time-of-flight, which is proportional to the mass of the individual molecules. After data processing, a spectrum is produced with relative intensity on the y-axis and mass/charge on the x-axis. Data acquired by the MassARRAY Analyzer is processed by MassARRAY Typer software, and then the SC2 Report software.

Table 1.5 Workflow

Step	See...
1. Software Setup	Chapter 2
• Import the assay design file. *Only required before the first time you run the panel.* • Create a virtual plate.	
2. Assay Protocol	Chapter 3
RNA extraction RT-PCR amplification SAP reaction iPLEX Pro extension reaction Water addition	
3. Data Acquisition on the MassARRAY System	
MassARRAY System with CPM 96	Chapter 4
MassARRAY System with CPM 384	Chapter 5
MassARRAY Analyzer 4 and Nanodispenser RS1000	Chapter 6
4. Generate Results Reports	Chapter 7

1.6 Samples and controls

Patient samples must be collected according to appropriate clinical guidelines. Positive and negative test controls must be included to accurately interpret patient test results.

Include the following controls on each plate:

Table 1.6 Controls

Control	Used to Monitor	Assays
Positive Control (Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 #102019)	RT-PCR reaction set up and reagent integrity	All five SARS-CoV-2 assays
Negative Control	Contamination during RNA extraction and reaction setup	All five SARS-CoV-2 assays MS2 assay
MS2 Control	RNA extraction	MS2 assay

1.7 Warnings and precautions

- For *In Vitro* Diagnostic Use pending FDA review.
- For prescription use only.
- Care must be taken to avoid mislabeling/misidentifying samples.
- Reduced sample input can adversely affect ability to detect SARS-CoV-2 virus.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Positive results are indicative of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases.
- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling samples. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Dispose of waste in compliance with local, state, and federal regulations.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- Fresh clean gloves must be worn in each area and must be changed before leaving that area.
- Do not pipette by mouth.
- Working with TRI reagent requires personal protective equipment (PPE) and a chemical fume hood with adequate ventilation.

- Prolonged exposure to phenol fumes or contact with skin can be hazardous and emergency medical attention should be initiated.
- Safety Data Sheets (SDS) are available on the Agena customer support portal, or by contacting Customer Support.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted.
- Do not use the kit or any kit components past the expiration date indicated on the kit carton label.
- In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
- Reagents must be stored as specified in Table 1.2 or by their manufacturer.
- Ensure all equipment utilized is calibrated and maintained according to manufacturer instructions.

1.8 Limitations

- The SARS-CoV-2 Panel is pending FDA review, and is for use in US laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.
- This device may not be able to differentiate newly emerging SARS-CoV-2 subtypes.
- Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of samples may hinder the ability of the assay to detect the target sequences.
- The performance of the SARS-CoV-2 Panel was established using nasopharyngeal swabs (NP) and bronchoalveolar lavage (BAL) samples. Oropharyngeal swabs are also considered acceptable sample types for use with the SARS-CoV-2 Panel, but performance has not been established.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- This test is a qualitative test and does not provide the quantitative value of detected organisms present.
- There is a risk of false positive values resulting from:
    - Cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
    - Cross-contamination during sample handling or preparation.
    - Cross-contamination between patient samples.

  - Sample mix up.
  - RNA contamination during product handling.
- There is a risk of false negative values due to:
  - The presence of sequence variants in the pathogen targets of the assay, procedural errors, amplification inhibitors in samples, or inadequate numbers of organisms for amplification.
  - Improper sample collection.
  - Sample mix up.
  - Degradation of the SARS-CoV-2 RNA during shipping/storage.
  - Sample collection does not collect SARS-CoV-2 RNA.
  - Using unauthorized extraction or assay reagents.
  - The presence of RT-PCR inhibitors.
  - Mutation in the SARS-CoV-2 virus.
  - Failure to follow instructions for use.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
- This test cannot rule out infections caused by other viral or bacterial pathogens not present on this panel.
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- This device has been evaluated for use with human sample material only.
- The performance of this device has not been evaluated for patients without signs and symptoms of infection.
- The performance of this device has not been evaluated for monitoring treatment of infection.

1.9 Symbols

Table 1.7 Symbols

REF	Catalog number		Temperature limits
LOT	Batch code		Manufacturer
	Use-by date	IVD	For in vitro diagnostic use
QTY	Quantity	Σ	Contains sufficient for <n> tests

Chapter 2

Software Setup

2.1 Import the assay design file

Note: The assay design file only needs to be imported once, prior to running the panel for the first time.

1. Locate the SC2 assay design file (*SC2_ADF.tsv*), at C/MassARRAY/Typer/bin/Reports/SC2 Report.
2. Open MassARRAY Typer Assay Editor.
3. Create a new assay project in the Database Browser by right clicking the root node and selecting **Project Administrator**.
4. Add a new Assay Project with an appropriate name. The new Assay Project will appear in the database browser. The SC2 assay design file will be stored in this project.
5. Right-click on the newly created Assay Project and select **Import Assay Group in Designer format...**
6. Remove the checkmarks next to **Design Summary** and **SNP Group**. Make sure that there is a checkmark next to **Assay Group**.
7. Click the **Browse** button next to Assay Group. In the Open window, select **All Files** in the **Files of type:** field.
8. Navigate to the folder containing the SC2 assay design file (*SC2_ADF.tsv)*, select it, and click **Open**.
9. Click the **Import** button to import the file.

2.2 Create a virtual plate

Note: Plate names and sample names should all be unique.

Create a sample group

Note: Each plate contains one positive control sample and one negative control sample. In order for the software to recognize the controls, "PC" must be the prefix for positive control samples, and "NC" must be the prefix for negative control samples (e.g., *PC_1*, *NC_1*).

1. Create a text file (.txt) of sample descriptions in a spreadsheet application such as Microsoft® Excel. The file must contain the sample ID in column A, and may contain a description in column B.
2. Create a sample customer and sample project in the database, if you have not already done so.
   a. Open the MassARRAY Typer Plate Editor software and click on the Sample tab.
   b. To create a sample customer, right-click the root-node and select **Add New Sample Customer**. Enter a sample customer ID and additional optional information and click **OK**. The new sample customer will appear in the Sample tab.
   c. To create a sample project, right-click the sample customer that the sample project will be under and select **Add New Sample Project**. Enter a sample project

ID and optional additional information and click **OK**. The new sample project will appear in the Sample tab.

3. Create a sample group.
   a. Right-click on the sample project that you want to add the sample group to and select **Add New Sample Group**.
   b. Enter a sample group ID and click the folder button in the toolbar to browse to the location of your sample group text file, and click **Open**, then click **OK**.

Create a Plate

1. Create a customer and project in the database, if you have not already done so.
   a. In the MassARRAY Typer Plate Editor software click on the Plate tab.
   b. To create a customer, right-click the root-node and select **New Customer**. Enter a customer ID and additional optional information and click **OK**. The new customer will appear in the Plate tab.
   c. To create a project, right-click the customer that the project will be under and select **New Project**. Enter a project ID and optional additional information and click **OK**. The new project will appear in the Plate tab.
2. Create a plate.
   a. Right-click on the project that you want to add the plate to and select **New Plate**.
   b. Enter a unique plate ID and select the plate type (96- or 384-well) and click **OK**. The new plate will appear in the Plate tab and a plate layout will be created automatically, based on the plate type specified.

Apply Assays to the Plate

1. In the Plate tab, select the plate that was just created.
2. Select the Assay tab and locate the SC2 assay design file you imported earlier (*SC2_ADF.tsv*).
3. In the plate layout, select the wells of interest.
4. In the Assay tab, right-click the plex or assay that you want to assign to the selected wells and select **Add plex** or **Add assay**.

Apply Samples to the Plate

1. Click on the Sample tab.
2. In the plate layout, select the wells of interest.
3. Right-click the sample or sample group that you want to assign to the selected wells and select **Apply Samples from Group** or **Add Sample**.
4. Select **File** > **Save** from the toolbar.

3.1 Lab areas and plate layout

The laboratory space should include three separate (non-contiguous) work areas to prevent contamination of PCR products. Table 3.1 shows the activities that are conducted in each area.

Table 3.1 Lab Area Activities

Lab Area	Activities
1	Isolation and preparation of RNA.
2	Pre-PCR preparation, including preparation of the RT-PCR cocktail, addition of the RT-PCR cocktail and RNA to the reaction plate, and preparation of the SAP and extension cocktails.
3	Positive control sample preparation. Thermocycling the reaction plate after addition of RT-PCR cocktail and RNA; addition of the SAP cocktail to the reaction plate and thermocycling; addition of the extension reaction cocktail to the reaction plate and thermocycling; desalting; nanodispensing; and data acquisition.

Include one positive control and one negative control on each plate.

3.2 RNA extraction

IMPORTANT

Perform this procedure in **Lab Area 1**.

WARNING

Be careful not to disturb the RNA pellet when discarding supernatant.

Preparation before each extraction

1. Thaw MS2 Control and samples on ice.
2. Vortex the tubes containing the sample at maximum speed for 1 minute.
3. Use 300 µL as the input sample in Step 3 below.

RNA Extraction

1. Determine the number of required reactions based on the number of patient samples to be processed, plus one negative control per extraction set.
2. Prepare fresh 75% ethanol solution using 100% absolute ethanol and nuclease-free water, sufficient for 1 mL per reaction, plus 10% overage.
3. Place 300 µL of sample in universal transport medium (or of nuclease-free water, for the negative control) into a new 1.5 mL tube.
4. Add 800 µL ice-cold TRI reagent and mix by pipetting and vortexing.

5. Add 10 µL of MS2 Control.
6. Incubate for 5 minutes at room temperature (20-25°C).
7. Add 200 µL chloroform and mix by vigorous shaking for 15 seconds.
8. Incubate for 5 minutes at room temperature (20-25°C).
9. Centrifuge for 15 minutes at 10,000 x g at 4°C.
10. Transfer the top aqueous layer (up to 600 µL) to a new tube using pipettor.

⚠ WARNING

Be careful not to transfer any of the other layers; instead, leave some of the top layer behind.

11. Add 2 µL of Glycoblue to tube and mix by pipetting.
12. Add 600 µL of ice-cold isopropanol (molecular biology grade) and invert 5 times to mix.
13. Incubate for 30 minutes at -20°C.
14. Centrifuge for 5 minutes at 10,000 x g at 4°C.
15. Visually check for the presence of the RNA pellet. If the pellet is not visualized, perform the following steps:
    a. Add 2 µL of Glycoblue to tube and mix by pipetting.
    b. Incubate for 10 minutes at -20°C.
    c. Centrifuge for 5 minutes at 10,000 x g at 4°C.
16. Discard supernatant using a pipettor.
17. Add 1 mL of ice-cold 75% ethanol and mix by gently tapping the tube.
18. Centrifuge for 5 minutes at 7,500 x g at 4°C.
19. Discard supernatant using a pipettor.
20. Centrifuge for 1 minute at 7,500 x g at 4°C.
21. Discard supernatant using a pipettor (P10 or P100/200).
22. Airdry for 10 minutes at room temperature (20-25°C).

⚠ WARNING

Insufficient evaporation of ethanol can cause inhibition of PCR.

23. Resuspend in 10 µL of nuclease-free water and incubate at 37°C for 10 minutes.

STOPPING POINT

Use RNA right away or store in sealed container at -80°C until needed.

3.3 Positive control sample preparation

IMPORTANT

Prepare the positive control sample in **Lab Area 3**.

Prepare the positive control by diluting the Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 ($1x10^6$ copies/µL) to a working stock of 16.7 copies/µL, as shown in Table 3.2. Make multiple aliquots at once and store at -80°C.

WARNING

Exercise caution when performing the serial dilutions. Risk of lab contamination is high due to the highly concentrated RNA control being used.

1. Prepare a 100-fold dilution by pipetting 990 µL of nuclease-free water into a microfuge tube, then adding 10 µL of SARS-CoV-2 RNA Control. Mix well, then centrifuge briefly.
2. Prepare a 10-fold dilution by pipetting 90 µL of nuclease-free water into a microfuge tube, then adding 10 µL of the step 1 dilution. Mix well, then centrifuge briefly.
3. Prepare a 10-fold dilution by pipetting 90 µL of nuclease-free water into a microfuge tube, then adding 10 µL of step 2 dilution. Mix well, then centrifuge briefly.
4. Pipette 83.3 µL of nuclease-free water into a microfuge tube, then add 16.7 µL of step 3 dilution. Mix well, then centrifuge briefly.

Table 3.2 Positive Sample Control Dilution

	Step	Final concentration (copies/µL)	Amount of SARS-CoV-2 RNA Control transferred (µL)	Source	Nuclease-free water	Final Volume (µL)
Dilutions	**1**	10,000	10	Stock	990	1,000
	2	1,000	10	Step 1	90	100
	3	100	10	Step 2	90	100
	4	16.7	16.7	Step 3	83.3	100

Label with correct copies/µL and store each dilution in aliquots at -80°C. When needed to make new positive controls, take one of the tubes and dilute as per table above, from the specific copy number down.

3.4 RT-PCR amplification

IMPORTANT

Prepare the RT-PCR cocktail and add cocktail and samples to the reaction plate in **Lab Area 2**. Thermocycle the RT-PCR reaction plate in **Lab Area 3**. Maintain an RNase-free environment and keep samples on ice during use. Make sure all reagents are thawed completely at room temperature and enzymes are kept on ice. Make sure reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept on ice.

1. Turn on the PCR thermal cycler with the program shown in Table 3.5, so that the heated cover can come to the correct operating temperature and there is no time delay between the RT-PCR reaction plate being prepared and cycling being started.

2. Prepare the RT-PCR cocktail in a 1.5 mL tube placed on ice or a cold block by adding reagents in the order in which they are listed in Table 3.3.
   Prepare more cocktail than the number of RT-PCR reactions to be performed. Either prepare for one or more extra reactions or use a percentage extra to ensure sufficient overage is present to overcome typical pipetting variation.

Table 3.3 RT-PCR Reaction

Reagent	Per Reaction (µL)
10X PCR Buffer	0.500
$MgCl_2$, 25 mM	0.400
dUTP/dNTP Mix	0.100
UNG (heat labile)	0.050
RNase Inhibitor	0.125
PCR Enzyme	0.200
MMLV Enzyme	0.125
SARS-CoV-2 PCR Primer	0.500
RT-PCR Cocktail Final Volume	**2.000**
Sample RNA/Positive Control/ Negative Control	3.000
RT-PCR Reaction Final Volume	**5.000**

3. Pulse vortex the tube briefly 3 times and briefly centrifuge.
4. Dispense 2.0 µL RT-PCR cocktail into each well of a new microtiter plate.
5. Add 3.0 µL of either sample RNA, positive control, or negative control to each well of the plate.

Table 3.4 Samples and Controls

Component	Volume per reaction		
	Sample reaction	Positive control reaction	Negative control reaction
RT-PCR Cocktail	2.0 µL	2.0 µL	2.0 µL
Purified sample RNA (from RNA Extraction section above)	3.0 µL	--	--
Positive control (from Positive Control Preparation section above)	--	3.0 µL	--
Purified negative control (from RNA Extraction section above)	--	--	3.0 µL
Total volume	**5.0 µL**	**5.0 µL**	**5.0 µL**

6. Seal the RT-PCR reaction plate, briefly pulse vortex 1-2 times, then centrifuge at 1000 x g for 15 seconds.
7. Visually inspect the individual wells from the bottom of the reaction plate to confirm uniform and adequate cocktail solution is present in every well before continuing.

8. Thermocycle the RT-PCR reaction plate using the conditions in Table 3.5. On a standard cycler with a ramp rate of approximately 3-4°C/s this program takes approximately 2 hours and 45 minutes.

Table 3.5 RT-PCR Thermal Cycling Conditions

Step		Temperature	Time	Number of Cycles
1	UNG incubation	25°C	5 minutes	1 cycle
2	RNA reverse transcription into cDNA	55°C	30 minutes	1 cycle
3	Polymerase activation	95°C	2 minutes	1 cycle
4	PCR to amplify specific fragments	95°C	30 seconds	45 cycles of steps 4-6
5		60°C	30 seconds	
6		72°C	1 minute	
7	Final extension	72°C	5 minutes	1 cycle
8	Sample preservation	10°C	--	Hold

STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

3.5 SAP reaction

IMPORTANT

Prepare the SAP cocktail in **Lab Area 2**. Add the SAP cocktail to the RT-PCR reaction plate and thermocycle the plate in **Lab Area 3**. Make sure all reagents are thawed completely and enzymes are kept on ice. Make sure all reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept on ice.

1. Prepare the SAP cocktail in a 1.5 mL tube on ice or a cold block as shown in Table 3.6. Prepare more cocktail than the number of SAP reactions to be performed. Either prepare for one or more extra reactions or use a percentage extra to ensure sufficient overage is present to overcome typical pipetting variation.

Table 3.6 SAP Cocktail

Reagent	Per reaction (µL)
HPLC-grade water	1.53
SAP Buffer	0.17
Shrimp Alkaline Phosphatase (SAP)	0.30
SAP Cocktail Final Volume	**2.00**

2. Pulse vortex the tube briefly 3 times and briefly centrifuge.
3. Centrifuge the RT-PCR reaction plate at 1000 x g for 15 seconds.
4. Dispense 2 µL of SAP cocktail into each well of the reaction plate.

5. Seal the reaction plate, briefly pulse vortex 1-2 times, then centrifuge at 1000 x g for 15 seconds.
6. Visually inspect the individual wells from the bottom of the reaction plate to confirm uniform and adequate solution is present in every well before continuing.
7. Thermocycle the reaction plate using the conditions in Table 3.7. On a standard cycler with a ramp rate of approximately 3-4°C/s this program takes approximately 45-50 minutes.

Table 3.7 SAP Cycling Conditions

Step		Temperature	Time	Number of Cycles
1	Dephosphorylation	37°C	40 minutes	1 cycle
2	Enzyme inactivation	85°C	5 minutes	1 cycle
3	Sample preservation	10°C	--	Hold

STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

3.6 iPLEX Pro extension reaction

IMPORTANT

Prepare the extension reaction cocktail in **Lab Area 2**. Add the extension reaction cocktail to the reaction plate and thermocycle the plate in **Lab Area 3**. Make sure all reagents are thawed completely and enzymes are kept on ice. Make sure all reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept on ice.

1. Prepare the extension cocktail in a 1.5 mL tube on ice or a cold block, as shown in Table 3.8. Prepare more cocktail than the number of extension reactions to be performed. Either prepare for one or more extra reactions per extension or use a percentage extra to ensure sufficient overage is present to overcome typical pipetting variation.

Table 3.8 Extension Cocktail

Reagent	Per reaction (µL)
HPLC-grade water	0.62
iPLEX Buffer Plus, GPR	0.20
iPLEX Termination Mix	0.20
iPLEX Pro Enzyme	0.04
SARS-CoV-2 Extend Primer	0.94
Extension Cocktails Final Volume	**2.00**

2. Pulse vortex the tube briefly 3 times and briefly centrifuge.
3. Centrifuge the reaction plate at 1000 x g for 15 seconds.
4. Dispense 2 µL of extension reaction cocktail into each well of the reaction plate.
5. Seal the reaction plate, briefly pulse vortex 1-2 times, then centrifuge at 1000 x g for 15 seconds.
6. Visually inspect the individual wells from the bottom of the reaction plate to confirm uniform and adequate solution is present in every well before continuing.
7. Thermocycle the reaction plate using the conditions in Table 3.9. On a standard cycler with a ramp rate of approximately 3-4°C/s this program takes approximately 2 hours and 35 minutes.

Table 3.9 Extension Thermal Cycling Conditions

Step		Temperature	Time	Number of Cycles	
1	Initial denaturation	95°C	30 seconds	1 cycle	
2	Denaturation	95°C	5 seconds	1 cycle	40 cycles of: 1 cycle of step 2 followed by 5 cycles of steps 3 and 4 (200 cycles total)
3	Annealing/Extension	52°C	5 seconds	5 cycles	
4	Denaturation	80°C	5 seconds		
5	Final extension	72°C	3 minutes	1 cycle	
6	Sample preservation	10°C	--	Hold	

STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

3.7 Water addition

1. Add HPLC-grade water to each well of the reaction plate.
   a. For 96-well plates, add 41 µL.
   b. For 384-well plates, add 16 µL.
2. Seal the plate and centrifuge at 1000 x g for 1 minute.

STOPPING POINT

If not proceeding directly to processing the plate on the MassARRAY System, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing more than 24 hours). Do not store for more than 2 weeks.

3.8 Data acquisition

Follow the data acquisition instructions in the chapter for the MassARRAY System you are using.

Table 3.10 Data Acquisition Instructions

System	Instructions
MassARRAY System with Chip Prep Module 96	Chapter 4
MassARRAY System with Chip Prep Module 384	Chapter 5
MassARRAY Analyzer 4 and Nanodispenser RS1000, 96- or 384-format	Chapter 6

Chapter 4

Data Acquisition on the MassARRAY System with Chip Prep Module 96

4.1 Create an input file

1. Double-click the Chip Linker icon on the desktop.
2. In the dialog box that appears, enter your username, password, and server.
3. Click **Connect**. The Chip Linker window appears.
4. Select a plate in the Chip Linker directory tree.
5. Select **iPLEX** as the terminator chemistry.
6. Select **Genotype+Area** for the process method.
7. Select **Nanodispenser 96 to 96** as the dispenser method.
8. Enter an experiment name.
9. Enter the SpectroCHIP Array barcode or other SpectroCHIP Array identifier.
10. Click **Add**. The input information appears in the Chip Linker table.
11. If a second SpectroCHIP Array will be processed, repeat step 4 to step 10 for the second SpectroCHIP Array.
12. Click **Create** to create an input XML file. This file will be selected for use when you set up the automatic run.

4.2 Prepare the instrument

1. Double-click the **Start All** icon to start MassARRAY Caller, Analyzer Control, Chip Prep Controller (CPC), and SpectroACQUIRE.
2. In the Status section in the Run Setup tab of SpectroACQUIRE check the Waste Tank, System Fluid, and Resin buttons; they should be green/Okay. If any are red (Waste Tank Full, System Fluid Empty, Resin Low or Empty) perform the necessary maintenance.
3. Click **Chip prep module Deck In/Out** at the top of the SpectroACQUIRE window. The deck will extend.
4. If there are SpectroCHIP Arrays in the completed chips position on the deck remove them.
5. Allow the calibrant to equilibrate to room temperature for 5 minutes (if it has been refrigerated) or 10 minutes (if it has been frozen). Pipette 75 µL of calibrant into the calibrant vial and place in the calibrant vial holder on the deck.
6. Load the SpectroCHIP Arrays.
    a. Orient the Chip holder so that the bevelled corner is at the top right.
    b. Open a new SpectroCHIP Array pouch and insert the new SpectroCHIP Array into the chip holder in position 1 (on the left); orient the SpectroCHIP Array such that the Agena logo and barcode are at the bottom. Make sure that the SpectroCHIP Array is properly seated so that the chip holder and SpectroCHIP Array surface are flush.

c. If you are processing two plates, insert another new SpectroCHIP Array into the chip holder in position 2 (on the right). If you are only processing one plate, place a previously completed SpectroCHIP Array in position 2, as the MassARRAY Analyzer requires both SpectroCHIP Array positions to be filled to function properly.

d. Place the full chip holder in the chip holder tray, new chips position, on the deck.

7. Load microtiter plates (MTPs). Up to two 96-well microtiter plates of analyte may be loaded.

WARNING

The Chip Prep Module is configured at installation for the particular plates you will be using. If at any point you wish to change the plates you are using, contact Agena Bioscience Customer Support to update instrument configuration. Using plates with different well depth without reconfiguration can damage the instrument or compromise assay performance.

a. Centrifuge the plates at 1000 x g for 1 minute.

b. Place the first MTP on MTP holder 1 (on the left). This corresponds to chip position 1. Orient the plate such that well A1 is in the front left corner.

c. If a second MTP is being processed, place it on MTP holder 2 (on the right). This corresponds to chip position 2.

8. Click **Chip prep module Deck In/Out** again to retract the deck.

4.3 Set up and start the run

WARNING

Confirm that instrument settings are correct, as shown in the tables below, prior to each run.

1. Select the **Run Setup** tab in SpectroACQUIRE.
2. In the Experiment Setup section:
   a. Under MTP 1, click on the browse button next to Experiment Name and select the XML input file created earlier.
   b. In the Wells to Process field, select **Automatic**.
   c. Repeat steps 1 and 2 for MTP 2 if running two plates. Otherwise, select **None** in the Experiment Name and Wells to Process fields for MTP 2.
   d. Enter the settings for the SpectroCHIP type being used, as shown in Table 4.1.

Table 4.1 Experiment Setup Settings

	SpectroCHIP CPM-96	SpectroCHIP-96
Use Autotune	Selected	Selected
Start Dispense Condition	600	650
Resin Volume	13	13
Sample Volume	n/a	10

3. In the Analyzer Setup section:
   a. Select **Tools** > **Load Parameters** on the SpectroACQUIRE toolbar, then select the appropriate parameter file based on the SpectroCHIP type. Acquisition parameters will automatically populate.
   b. Make sure all settings in this section are as shown in Table 4.2.

Table 4.2 Analyzer Setup Settings

	SpectroCHIP CPM-96	SpectroCHIP-96
Parameter file name	iPLEX_CPM.par	iPLEX.par
Shots (n)	30	20
Maximum acquisitions	9	9
Minimum good spectra	5	5
Maximum good spectra	5	5
Turn Off HV After Analysis	Selected	Selected
Analyze Calibrant Pads	Selected	Selected
Filter Saturated Shots	Selected	Selected
Chip Type	SpectroCHIP CPM-96	SpectroCHIP-96

4. In the Chip Prep Module Setup section:
   a. Check the **Normal Operation** box.
   b. Check **MTP Barcodes Required**, if desired.
   c. Select **iPLEX** in the Chemistry drop-down menu.
5. In the Temperature Control section:
   a. If the MTP and calibrant will not be removed from the instrument shortly after the run is completed, select the **MTP Cool** box, and check the **Auto** box.
   b. If using a SpectroCHIP CPM-96, check the **Chip Heat** box and enter **30** in the Setpoint field.
6. If desired, enter your email and check the **When Chip prep module is finished** and **When MA4 is finished boxes** to receive email notifications.
7. Click **Start Chip prep module** to start the run.

4.4 Remove plates, calibrant, and SpectroCHIP Arrays when run is complete

1. Once the run is completed, click **Chip prep module Deck In/Out** to move the deck out and remove the MTPs and calibrant vial. Store remaining calibrant refrigerated or frozen for future use.
2. Click **Remove Old Chips from MA4** in the Run Setup tab. The instrument will move the completed SpectroCHIP Arrays from the MassARRAY Analyzer to the completed chips position on the Chip Prep Module deck, and then extend the deck so you may remove the SpectroCHIP Arrays from the completed chips position.

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Chapter 5

Data Acquisition on the MassARRAY System with Chip Prep Module 384

5.1 Create an input file

1. Double-click the Chip Linker icon on the desktop.
2. In the dialog box that appears, enter your username, password, and server.
3. Click **Connect**. The Chip Linker window appears.
4. Select a plate in the Chip Linker directory tree.
5. Select **iPLEX** as the terminator chemistry.
6. Select **Genotype+Area** for the process method.
7. Select **Nanodispenser 384 to 384** as the dispenser method.
8. Enter an experiment name.
9. Enter the SpectroCHIP Array barcode or other SpectroCHIP Array identifier.
10. Click **Add**. The input information appears in the Chip Linker table.
11. If a second SpectroCHIP Array will be processed, repeat step 4 to step 10 for the second SpectroCHIP Array.
12. Click **Create** to create an input XML file. This file will be selected for use when you set up the automatic run.

5.2 Prepare the instrument

1. Double-click the **Start All** icon to start MassARRAY Caller, Analyzer Control, Chip Prep Controller (CPC), and SpectroACQUIRE.
2. In the Status section in the Run Setup tab of SpectroACQUIRE check the Waste Tank, System Fluid, and Resin buttons; they should be green/Okay. If any are red (Waste Tank Full, System Fluid Empty, Resin Low or Empty) perform the necessary maintenance.
3. Click **Chip prep module Deck In/Out** at the top of the SpectroACQUIRE window. The deck will extend.
4. If there are SpectroCHIP Arrays in the completed chips position on the deck remove them.
5. Allow the calibrant to equilibrate to room temperature for 5 minutes (if it has been refrigerated) or 10 minutes (if it has been frozen). Pipette 75 µL of calibrant into the calibrant vial and place in the calibrant vial holder on the deck.
6. Load the SpectroCHIP Arrays.
    a. Orient the Chip holder so that the bevelled corner is at the top right.
    b. Open a new SpectroCHIP Array pouch and insert the new SpectroCHIP Array into the chip holder in position 1 (on the left); orient the SpectroCHIP Array such that the Agena logo and barcode are at the bottom. Make sure that the SpectroCHIP Array is properly seated so that the chip holder and SpectroCHIP Array surface are flush.

c. If you are processing two plates, insert another new SpectroCHIP Array into the chip holder in position 2 (on the right). If you are only processing one plate, place a previously completed SpectroCHIP Array in position 2, as the MassARRAY Analyzer requires both SpectroCHIP Array positions to be filled to function properly.

d. Place the full chip holder in the chip holder tray, new chips position, on the deck.

7. Load microtiter plates (MTPs). Up to two 384-well microtiter plates of analyte may be loaded.

WARNING

The Chip Prep Module is configured at installation for the particular plates you will be using. If at any point you wish to change the plates you are using, contact Agena Bioscience Customer Support to update instrument configuration. Using plates with different well depth without reconfiguration can damage the instrument or compromise assay performance.

a. Centrifuge the plates at 1000 x g for 1 minute.

b. Place the first MTP on MTP holder 1 (on the left). This corresponds to chip position 1. Orient the plate such that well A1 is in the front left corner.

c. If a second MTP is being processed, place it on MTP holder 2 (on the right). This corresponds to chip position 2.

8. Click **Chip prep module Deck In/Out** again to retract the deck.

5.3 Set up and start the run

WARNING

Confirm that instrument settings are correct, as shown in the tables below, prior to each run.

1. Select the **Run Setup** tab in SpectroACQUIRE.

2. In the Experiment Setup section:

a. Under MTP 1, click on the browse button next to Experiment Name and select the XML input file created earlier.

b. In the Wells to Process field, select **Automatic**.

c. Repeat steps 1 and 2 for MTP 2 if running two plates. Otherwise, select **None** in the Experiment Name and Wells to Process fields for MTP 2.

d. Enter the settings as shown in Table 5.1.

Table 5.1 Experiment Setup Settings

	SpectroCHIP CPM-384
Use Autotune	Selected
Start Dispense Condition	Default (350)
Resin Volume	10
Sample Volume	n/a

3. In the Analyzer Setup section:

a. Select **Tools** > **Load Parameters** on the SpectroACQUIRE toolbar, then select the appropriate parameter file based on the SpectroCHIP type. Acquisition parameters will automatically populate.

b. Make sure all settings in this section are as shown in Table 5.2.

Table 5.2 Analyzer Setup Settings

	SpectroCHIP CPM-384
Parameter file name	iPLEX_CPM.par
Shots (n)	30
Maximum acquisitions	9
Minimum good spectra	5
Maximum good spectra	5
Turn Off HV After Analysis	Selected
Analyze Calibrant Pads	Selected
Filter Saturated Shots	Selected
Chip Type	SpectroCHIP CPM-384

4. In the Chip Prep Module Setup section:
   a. Check the **Normal Operation** box.
   b. Check **MTP Barcodes Required**, if desired.
   c. Select **iPLEX** in the Chemistry drop-down menu.
5. In the Temperature Control section:
   a. If the MTP and calibrant will not be removed from the instrument shortly after the run is completed, select the **MTP Cool** box, and check the **Auto** box.
   b. Check the **Chip Heat** box and enter **30** in the Setpoint field.
6. If desired, enter your email and check the **When Chip prep module is finished** and **When MA4 is finished boxes** to receive email notifications.
7. Click **Start Chip prep module** to start the run.

5.4 Remove plates, calibrant, and SpectroCHIP Arrays when run is complete

1. Once the run is completed, click **Chip prep module Deck In/Out** to move the deck out and remove the MTPs and calibrant vial. Store remaining calibrant refrigerated or frozen for future use.
2. Click **Remove Old Chips from MA4** in the Run Setup tab. The instrument will move the completed SpectroCHIP Arrays from the MassARRAY Analyzer to the completed chips position on the Chip Prep Module deck, and then extend the deck so you may remove the SpectroCHIP Arrays from the completed chips position.

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Chapter 6

Data Acquisition on the MassARRAY Analyzer 4 and Nanodispenser RS1000, 96- or 384-format

6.1 Desalt the analyte

1. Centrifuge the reaction plate at 3200 x g for 1 minute.
2. Spoon 3 scoops of Clean Resin on a clean, dry dimple plate (96/15 mg plate or 384/6 mg plate as appropriate).
3. Spread out the Clean Resin on the dimple plate using the scraper, making sure the resin settles evenly into all wells.
4. Let resin plate dry for 10 minutes at room temperature.
5. Seal the plate and centrifuge at 3200 x g for 1 minute.
6. To add dried Clean Resin to each well:
   a. Gently invert the sample plate on top of the dimple plate, making sure that the plate wells are aligned over the resin samples.
   b. Keep the sample and dimple plates pressed together, and invert both plates so that the dimple plate is on top of the sample plate.
   c. Gently tap the dimple plate and let the resin fall into the sample wells.
   d. Remove dimple plate.
7. Seal the plate and rotate for at least 15 minutes. The rotator must rotate the microplate 360° around its long axis.

6.2 Prepare the Nanodispenser RS1000

1. Turn on the Nanodispenser RS1000 and log in.
2. If this is the first run of the day, complete the daily maintenance procedures.
   a. Perform daily pin maintenance by soaking the pins in 100% ethanol for 30 minutes.
   b. Replace 100% ethanol with 50% ethanol prior to beginning dispensing run.
3. Check tank status.
   a. Tap **STATUS** button at top of Main Menu screen.
   b. Tap **Page 2** tab.
   c. Check status of the supply and waste tanks. Add supply tank water and drain waste tank if indicated.

6.3 Set up the dispensing run

1. Load a method file.
   a. From the Main Menu, tap **TRANSFER**.
   b. Tap **METHODS**.

c. Tap **OPEN**.

d. Tap **96 MTP to 96 SpectroCHIP Array** or **384 MTP to 384 SpectroCHIP Array**, as appropriate.

2. Select setup parameters on the Method screen.

a. Tap to select the positions on the SCOUT plate that will contain SpectroCHIP Arrays.

b. Select the **Enable** checkbox under Sample Tracking to enable sample tracking, if desired.

c. Select the **Enable** checkbox under Auto tuning.

d. Enter **12** in the Target Volume field.

e. Select the **Enable** checkbox under Volume Check.

f. Enter **8** in the Lower Limit box.

g. Enter **20** in the Upper Limit box.

3. Tap the **cleaning** tab on the Method screen and verify that all cleaning steps are check marked in the Cleaning Setup box.

4. Tap the **aspirate/dispense** tab on the Method screen.

a. Under dispense settings, enter **100** for the dispense speed.

b. Under calibrant, enter **100** for the dispense speed.

c. Under operation, select **analyte & calibrant**.

6.4 Set up the instrument deck

1. Fill the calibrant reservoir.

a. Allow the calibrant to equilibrate to room temperature for 5 minutes (if refrigerated) or 10 minutes (if frozen).

b. Tap the **PARK** button.

c. Open the main door.

d. Fill the calibrant reservoir with 60 µL calibrant using a pipette.

2. Load the microtiter plates.

a. Open the main door.

b. If using a non-skirted 96-well MTP, place the MTP on the 96-well fully skirted plate base.

c. Place the first MTP on plate holder 1 (on the left), orienting it so that well A1 is located at the front left corner of the plate holder.

d. If there is a second MTP, place it on plate holder 2 (on the right).

3. Load the SpectroCHIP Arrays.

a. Remove the SCOUT plate from the deck, if it is not already removed.

b. Orient the SCOUT plate so that the bevelled corners are at the right.

c. Insert the SpectroCHIP Arrays into the SCOUT plate positions that were specified earlier. Orient the SpectroCHIP Arrays so that the Agena Bioscience logo is at the front. Make sure each SpectroCHIP Array is seated flat in its position and flush with the front-left corner.

d. Place the SCOUT plate back onto the deck, placing the left edge of the SCOUT plate down first, against the alignment post, and then pressing down the right edge until the spring-loaded positioners engage firmly against the plate.

4. Close the main door.
5. Tap **HOME**.

6.5 Start the nanodispensing run

1. Tap **BACK** on the Method screen.
2. Tap **Apply**.
3. Tap the **run** button.
4. Tap **OK** in the rinse station preparation window.
5. If the rinse station operates correctly (if water flows out of the rinse station chimneys), tap **YES**.

6.6 Remove plates, calibrant, and SpectroCHIP Arrays when run is complete

1. Tap the **BACK** button on the Transfer screen.
2. Tap **PARK**.
3. Open the main door.
4. Pull the spring-loaded positioners away from the SCOUT plate and lift it off the deck.
5. Remove the SpectroCHIP Arrays using tweezers and place them in the SpectroCHIP Array carriers for the MassARRAY Analyzer.
6. Remove the MTPs from the plate holders and discard or store for future use.
7. Close the main door.
8. Tap **HOME**.
9. Open the main door.
10. Pipette out any remaining calibrant and return it to the calibrant storage container for future use. Store at -20°C.
11. Close the main door.
12. Tap **HOME**.

6.7 Set up the acquisition run on the MassARRAY Analyzer 4

1. Double-click the Analyzer 4 instrument icon to launch the software.
2. Click the **Probe Sample In/Out** button in the SpectroACQUIRE toolbar to move the chip holder to the load position.
3. Open the sample chamber lid and remove the chip holder.
4. Remove any previously run SpectroCHIP Arrays from the chip holder.
5. Orient the child holder so that the bevelled corner is at the top right.
6. Open a new SpectroCHIP Array pouch and place the SpectroCHIP Array into the chip holder in position 1 (on the left), such that the Agena logo and barcode are at the bottom.

7. If running two SpectroCHIP Arrays, insert the second chip in the chip holder in position 2 (on the right). If only running one SpectroCHIP Array, place a previously run SpectroCHIP Array in position 2.
8. Place the loaded chip holder in the Analyzer 4 sample chamber. Firmly press down on the sample chamber lid.
9. Click **Probe Sample In/Out**.

6.8 Create an input file

1. Double-click the Chip Linker icon on the desktop.
2. In the dialog box that appears, enter your username, password, and server.
3. Click **Connect**. The Chip Linker window appears.
4. Select a plate in the Chip Linker directory tree.
5. Select **iPLEX** as the terminator chemistry.
6. Select **Genotype+Area** for the process method.
7. Select **Nanodispenser 96 to 96** or **Nanodispenser 384 to 384** as the dispenser method.
8. Enter an experiment name.
9. Enter the SpectroCHIP Array barcode or other SpectroCHIP Array identifier.
10. Click **Add**. The input information appears in the Chip Linker table.
11. If a second SpectroCHIP Array will be processed, repeat step 4 to step 10 for the second SpectroCHIP Array.
12. Click **Create** to create an input XML file. This file will be selected for use when you set up the Analyzer run.

6.9 Set up and start the Analyzer run

1. Select the **Run Setup** tab in SpectroACQUIRE.
2. Next to Chip 1, click on the browse button and select the XML input file created earlier.
3. Repeat for Chip 2 if acquiring data from a second SpectroCHIP Array.
4. Select **Tools** > **Load Parameters** on the SpectroACQUIRE toolbar and select the **iPLEX.par** parameter file, then click **Open**.
5. Ensure that the acquisition parameters as are shown in Table 6.1.

Table 6.1 Acquisition Parameters

Parameter	Setting
Shots (n)	20
Maximum acquisitions	9
Minimum good spectra	5
Maximum good spectra	5

WARNING

Confirm acquisition parameter settings prior to each run.

6. Select the **Filter Saturated Shots** checkbox.
7. Select the **Use Calibration Wells** and **Auto Teach Geometry** checkboxes.
8. Select **SpectroCHIP-96** or **SpectroCHIP-384** in the Chip Type menu.
9. Select the **Turn off HV After Last chip is complete** checkbox.
10. Click **Barcode Report** in the Automatic Run Setup tab.
11. Check that the status of each SpectroCHIP Array is **Found**.
12. Click **Close**.
13. If any errors were found, correct them, and perform another barcode report.
14. Select the **Automatic Run** tab.
15. Click the **Start Autorun** button.

6.10 Remove SpectroCHIP Arrays when run is complete

1. Select the **Automatic Run** tab.
2. Press the manual stage control button on the front of the Analyzer 4 to extend the target.
3. Open the sample chamber lid and take out the chip holder. Remove the SpectroCHIP Arrays from the chip holder and place the chip holder back into the chip carrier.
4. Press the manual stage control button to retract the chip carrier.

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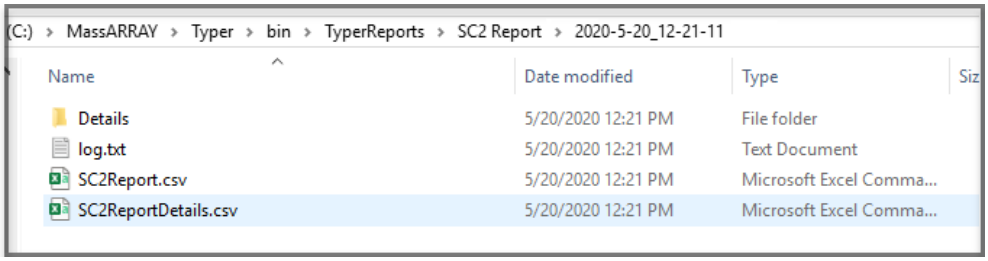
7.1 Analyze the data

1. Open MassARRAY Typer Analyzer and in the Project Explorer pane double click on the SpectroCHIP Arrays of interest. The SpectroCHIP Arrays will be added to the Chip List.
2. Load the SpectroCHIP Arrays by checking the box next to the SpectroCHIP Array names in the Chip List.
3. Select **File** > **Reports** > **SC2 Report-v1** in the MassARRAY Typer Analyzer menu bar.

When the report is complete, the *SC2ReportDetails.csv* will automatically open, and the results will be made available in a date- and time-stamped folder in the Typer/bin/TyperReports/SC2 Report folder.



7.2 Interpretation of the results

Interpretation of the results is performed by the Agena Bioscience SC2 Report software.

Quality control and validity of results

One negative control and one positive control are processed with each run.

Validation of results is performed automatically by the Agena Bioscience SC2 Report software based on performance of the positive and negative controls.

Table 7.1 Result Interpretation

SARS-CoV-2 Targets	MS2	QC Status	Result	Recommended Action
≥ 2 SARS-CoV-2 targets detected	Detected	PASS	Detected	Report results
≥ 2 SARS-CoV-2 targets detected	NotDetected	WARNING	Detected	Report results
< 2 SARS-CoV-2 targets detected	Detected	PASS	NotDetected	Report results
< 2 SARS-CoV-2 targets detected	NotDetected	FAIL	Invalid	Repeat test

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8.1 Limit of detection (LoD)

This LoD study established the lowest SARS-CoV-2 viral concentration (genomic copy equivalents or GCE) that can be detected by the Agena Bioscience SARS-CoV-2 Panel at least 95% of the time. Bronchoalveolar lavage (BAL) clinical samples were collected before October 2019. More recent clinical samples from nasopharyngeal (NP) swabs were collected, pooled, and tested negative using Hologic® Panther Fusion® SARS-CoV-2 Assay and confirmed using Agena Bioscience SARS-CoV-2 Panel.

Samples were spiked with synthetic SARS-CoV-2 RNA (Twist Bioscience, #102019) at several concentrations and processed through the Agena Bioscience SARS-CoV-2 Panel workflow on the MassARRAY System with Chip Prep Module 96. A three-phase approach was used to determine the LoD for each sample type. Phases I and II determined the LoD (Table 8.1 and Table 8.2), which was confirmed in Phase III by testing 20 replicates (Table 8.3).

Table 8.1 LoD Determination Study Results (BAL Samples)

Effective Concentration (copy/µL)	# Positive/Total Replicates	% Positive
0	0/3	0
0.00625	0/3	0
0.0125	0/3	0
0.025	1/3	33
0.05	2/3	67
0.1	2/3	67
0.2	3/3	100
0.4	3/3	100

Table 8.2 LoD Determination Study Results (NP Samples)

Effective Concentration (copy/µL)	# Positive/Total Replicates	% Positive
0	0/3	0
0.00625	1/3	33
0.0125	1/3	33
0.025	2/3	67
0.05	1/3	33
0.1	1/3	33
0.2	2/3	67
0.4	3/3	100

Table 8.3 LoD Confirmation Study Results

Effective Concentration (copy/µL)	# Positive/ Total Replicates	% Positive	Effective Concentration (copy/µL)	# Positive/ Total Replicates	% Positive
BAL Samples			NP Samples		
0.3	20/20	100	0.4	19/20	95

8.2 Inclusivity

Homology verification was carried out using a consensus sequence from 2,661 complete SARS-CoV-2 genomes (NCBI; May 20, 2020). A multiple sequence alignment (MAFFT version 7) was carried out to generate the consensus sequence, which was used to determine conserved regions of the virus. Subsequently all assay components (two PCR primers and a probe) were aligned to the conserved regions. All assay components exhibit 100% sequence homology to conserved SARS-CoV-2 regions except for the forward PCR primer for the SC2_N2 assay. According to the sequence data as of May 20, 2020, 8% of the SARS-CoV-2 sequences have a three-nucleotide mismatch with the first three 5' end nucleotides of the SC2_N2 forward PCR primer (22 nucleotide length). This results in the 86% PCR primer homology for the 8% of SARS-CoV-2 population and the 98.9% weighted homology. The mismatch is located at the 5' end of the PCR primer and does not affect the test performance.

8.3 Cross-reactivity

In silico cross-reactivity analysis was carried out with NCBI nucleotide BLAST against a list of FDA-recommended cross-reactivity organisms (Table 8.4). The analysis was performed using the May 22, 2020 version of the microbial NCBI database. Components of three assays exhibit greater than 80% homology to a listed evaluation specie.

- SC2_N1 assay. Two SC2_N1 assay components exhibit >80% *in silico* homology to a listed organism (SARS-coronavirus). However, the cross-reactive assay components are not the PCR primers, but a combination of one PCR primer and the probe. The forward primer showed 82% homology while the reverse primer showed 75% homology and the probe showed 94% homology to SARS-coronavirus. The SC2_N1 reverse primer shows low homology; therefore the risk of non-specific PCR amplification of SARS-coronavirus is low. This is confirmed by the NCBI Primer BLAST. Furthermore, reverse transcription of RNA into DNA requires high reverse PCR primer homology. Low homology of the reverse primer further reduces non-specific amplification.
- SC2_N2 assay. One SC2_N2 assay component exhibits >80% homology to a listed organism (SARS-coronavirus). The forward primer showed 91% homology while the reverse primer showed 68% homology and the probe showed 55% homology to SARS-coronavirus. The SC2_N2 reverse primer and probe show low homology; therefore the risk of non-specific PCR amplification and probe extension of SARS-coronavirus is low. This is confirmed by the NCBI Primer BLAST. Furthermore, reverse transcription of RNA into DNA requires high reverse PCR primer homology. Low homology of the reverse primer further reduces non-specific amplification.
- SC2_ORF1ab assay. One SC2_ORF1ab assay component exhibits >80% homology to a listed organism (SARS-coronavirus). The probe showed 88% homology while the forward primer showed 76% and the reverse primer showed 37% homology to SARS-coronavirus. The SC2_ORF1ab forward and reverse primers show low homology; therefore the risk of non-specific PCR amplification of SARS-coronavirus is low. This is confirmed by the NCBI Primer BLAST. Furthermore, reverse transcription of RNA into DNA requires high reverse PCR primer homology. Low homology of the reverse primer further reduces non-specific amplification.

Table 8.4 Organism List Used for BLAST Similarity Evaluation

High priority pathogens from the same genetic family	High priority organisms likely in circulating areas
Human coronavirus 229E	Adenovirus (e.g., C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g., EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Staphylococcus salivarius

8.4 Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the Agena Bioscience SARS-CoV-2 Panel, using clinical samples from nasopharyngeal (NP) and contrived bronchoalveolar lavage (BAL) samples. The BAL clinical samples were collected before October 2019 and NP clinical samples were obtained from Boca Biolistics (#C0040-0001).

Bronchoalveolar lavage samples

A total of 30 contrived positive samples were tested as well as 30 negative samples. Samples were contrived by spiking known concentrations of synthetic SARS-CoV-2 RNA (Twist Bioscience, #102019). The samples were randomized, blinded, and tested with the Agena Bioscience SARS-CoV-2 Panel. Twenty of the samples were spiked at 0.6 copies/µL, five at 1.2 copies/µL, and five at 2.4 copies/µL. Results are shown in Table 8.5.

Table 8.5 Clinical Evaluation Study Summary (BAL Samples)

Effective Concentration (copy/µL)	# Positive/Total Replicates	% Positive
0 copies/µL	0/30	0%
0.6 copies/µL	20/20	100%
1.2 copies/µL	5/5	100%
2.4 copies/µL	4/5*	80%

*The RNA extraction for the false negative result for the 2.4 copies/µL sample was retested at 2-fold and 4-fold dilution of the RNA extraction and both results were "Detected." This is indicative of RT-PCR inhibition due to insufficient alcohol removal from last wash step of RNA extraction.

Nasopharyngeal samples

A total of 30 individual positive clinical NP samples and 60 negative clinical NP samples characterized by the Hologic Panther Fusion SARS-CoV-2 Assay (Comparator Test) were tested. The samples were randomized, blinded, and tested with the Agena Bioscience SARS-CoV-2 Panel. Three samples were invalid due to RNA extraction and reaction setup control not being detected.

The positive percent agreement (PPA) was 97% and the negative percent agreement (NPA) was 72% as shown in Table 8.6. Discordant analysis for the 16 false positive and 1 false negative was performed with the Bio-Rad SARS-CoV-2 ddPCR Kit (#12008202/1864021) and these results are show in Table 8.7. The Agena Bioscience SARS-CoV-2 Panel had a 94% agreement with Bio-Rad SARS-CoV-2 ddPCR test, resolving 15 of 16 false positives and 1 of 1 false negatives, as shown in Table 8.8.

Table 8.6 PPA and NPA Results of the Agena Bioscience SARS-CoV-2 Panel Against Comparator Test (NP Samples)

		Comparator Test	
		Detected	Not Detected
Agena Bioscience SARS-CoV-2 Panel	Detected	29	16
	Not Detected	1	41
	Total	30	57
PPA: 97%; NPA: 72%			

Table 8.7 Description of Excluded and Discordant Cases (NP Samples)

Sample ID	Agena Bioscience SARS-CoV-2 Test	Comparator Test (Hologic Panther Fusion EUA Test)	Adjudication Test (Bio-Rad SARS-CoV-2 Test)
M15	Not Detected	Detected	Not Detected
M34	Detected	Not Detected	Detected
M35	Detected	Not Detected	Detected
M36	Detected	Not Detected	Detected
M37	Detected	Not Detected	Detected
M40	Detected	Not Detected	Detected
M43	Detected	Not Detected	Detected
M45	Detected	Not Detected	Detected
M48	Detected	Not Detected	Detected
M50	Detected	Not Detected	Detected
M51	Detected	Not Detected	Detected
M52	Detected	Not Detected	Not Detected
M58	Detected	Not Detected	Detected
M59	Detected	Not Detected	Detected
M91	Invalid	Not Detected	N/A
M92	Detected	Not Detected	Detected
M94	Invalid	Not Detected	N/A
M95	Detected	Not Detected	Detected
M104	Invalid	Not Detected	N/A
M105	Detected	Not Detected	Detected
M106	Detected	Not Detected	Detected
M112	Detected	Not Detected	Detected
M117	Detected	Not Detected	Detected
M119	Detected	Not Detected	Detected

Note: Samples M93, M96 through M103, M107 through M111, M113 through M116, M118, and M120 are *Not Detected* by all three tests.

Table 8.8 Summary of Test vs. Comparator vs. Adjudication (NP Samples)

Test	Comparator		Adjudication		
Agena Bioscience SARS-CoV-2 Panel (0.4 cps/µL)	Hologic Panther Fusion SARS-CoV-2 Assay (0.01 $TCID_{50}$/mL)	Total Subjects	Bio-Rad SARS-CoV-2 ddPCR Kit (0.6 cps/µL)		
			Detected	Not Detected	Not Tested
Detected	Detected	29	N/A	N/A	29
Detected	Not Detected	16	15	1	N/A
Not Detected	Detected	1	0	1	N/A
Not Detected	Not Detected	41	4	20	17
Invalid	Not Detected	3	N/A	N/A	3
	Total	**90**	**N/A**	**N/A**	**N/A**

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Chapter 9

Support

9.1 Customer support

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9.2 Related documentation

Table 9.1 Related User Documentation

Document	Document #
MassARRAY System with Chip Prep Module 96 User Guide	USG-CUS-069
MassARRAY System with Chip Prep Module 384 User Guide	USG-CUS-097
MassARRAY Analyzer 4 User Guide	USG-CUS-034
MassARRAY Nanodispenser RS1000 v2.1 User Guide	USG-CUS-059
MassARRAY Typer User Guide	USG-CUS-027