



# MassARRAY<sup>®</sup> System: Recommended Lab Equipment and Set-up

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## OVERVIEW

The purpose of this document is to provide recommendations for optimal lab set up and equipment for use with the Agena Bioscience MassARRAY<sup>®</sup> System. These recommendations are applicable for the MassARRAY Analyzer 4 with Chip Prep Module and the MassARRAY Analyzer 4 with Nanodispenser RS1000 (96, 384, and 96/384 formats), for iPLEX<sup>®</sup> Pro, iPLEX Gold, iPLEX HS, UltraSEEK<sup>®</sup>, and MassCLEAVE<sup>™</sup> chemistries. Though equivalent equipment from other manufacturers may be used, please consult with your Agena Bioscience representative before purchasing equivalent items.

## LAB SET UP

It is strongly recommended to create three separate lab areas to prevent cross-contamination and sample handling errors during processing. Each area should have its own equipment.

Lab Area	Activities
Area 1	Isolation, dilution, and quantitation of DNA. Isolation of RNA. cDNA synthesis. MassCLEAVE: bisulfite treatment.
Area 2	Pre-PCR preparation, including preparation of PCR cocktails and addition of PCR cocktail and DNA to the reaction plate. Preparation of the SAP and extension cocktails.
Area 3	Thermocycling the reaction plate after addition of PCR cocktails, addition of SAP and extension cocktails or T cleavage/RNase A cocktail to reaction plate and thermocycling. Water addition, desalting, nanodispersing and data acquisition. UltraSEEK: bead conditioning and capture, cleaning, and elution steps.



## CHECKLIST

Use this checklist to ensure the required equipment is available in the pre- and post-PCR areas prior to instrument installation and training on the MassARRAY System. This checklist focuses on material required for reaction set up and MassARRAY processing and does not include the equipment required for DNA extraction and quantification.

Equipment	Pre-PCR Area	Post-PCR Area
384-well MTP ( <i>384 systems only</i> )	<input type="checkbox"/> Pre-PCR	N/A
96-well MTP	<input type="checkbox"/> Pre-PCR	N/A
Centrifuge - Mini Tube	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Centrifuge - Plate	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
DNA Away	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Ethanol ( <i>RS1000 Nanodispenser only</i> )	N/A	<input type="checkbox"/> Post-PCR
Freezer	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Microtubes 1.5 mL	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Mini Cooler	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Multi-channel Pipettes & Filtered Tips	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
NaOH ( <i>RS1000 Nanodispenser only</i> )	N/A	<input type="checkbox"/> Post-PCR
HPLC-grade Water	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
PCR Strip Tubes	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
PCR Workstation	<input type="checkbox"/> Pre-PCR	N/A
Plate Rotator	N/A	<input type="checkbox"/> Post-PCR
Plate Sealing Roller Tool	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Plate Seals	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Reagent Reservoirs	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Refrigerator	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Single Channel Pipettes & Filtered Tips	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Thermocycler	<input type="checkbox"/> Pre-PCR ( <i>for cDNA synthesis and methylation panels only</i> )	<input type="checkbox"/> Post-PCR
Tween (optional)	N/A	<input type="checkbox"/> Post-PCR
Type 1 or Deionized Water	N/A	<input type="checkbox"/> Post-PCR
Vortex	<input type="checkbox"/> Pre-PCR	<input type="checkbox"/> Post-PCR
Isopropanol ( <i>Chip Prep Module only</i> )	N/A	<input type="checkbox"/> Post-PCR
Single Tube Magnetic Stand ( <i>UltraSEEK only</i> )	N/A	<input type="checkbox"/> Post-PCR
96-well Plate Magnetic Stand ( <i>UltraSEEK only</i> )	N/A	<input type="checkbox"/> Post-PCR

The following sections of the document provide detailed information on optimal laboratory set up and required equipment.



## POSITIVE CONTROLS

### General Genotyping Controls

- [Coriell HAPMAP Collections](#) – Normal or wild type DNA, cystic fibrosis, PGx.

### Somatic Mutations

- [ATCC Tumor Cell Panels](#) – NIST DNA standards, purified DNA and cell lines
- [Horizon HDx Reference Standards](#) – Purified genomic DNA and FFPE sections
- [SeraCare Reference Standards](#)

### Controls & Custom Plasmids

- [Maine Molecular Quality Controls](#) – Cystic Fibrosis, PGx, FII, FV

## DNA/RNA EXTRACTION

DNA/RNA extraction should be performed in Lab Area 1. Ensure that you have the required ancillary equipment (pipettes, vortex, mini centrifuge, etc.) for DNA extraction from samples. We recommend the following kits for manual extraction.

### DNA Extraction

#### General Purpose Human Tissue, Serum, Plasma and Urine Samples

- Qiagen [QIAamp DNA Blood Mini Kit](#) – suitable for DNA extraction from blood.
- Qiagen [QIAamp Circulating Nucleic Acid Kit](#) – suitable for DNA from plasma/serum.
- Qiagen [DNeasy Blood & Tissue Kit](#) – suitable for a wide variety of samples.
- Invitex [PSP SalivaGene DNA Kit](#) (#1035200200), using SalivaGene Swab Comfort (#1035231100).
- Macherey-Nagel [NucleoSpin Tissue](#) (#740952), using Puritan [6" Sterile Standard Polyester Swab with Polystyrene Handle](#) (Puritan #25-8061PD).
- Qiagen [QIAamp DNA Mini Kit](#) (#51304/51306), using Puritan [6" Sterile Standard Polyester Swab with Polystyrene Handle](#) (Puritan #25-8061PD).
- Omega Bio-Tek [Mag-Bind® Viral RNA Xpress Kit](#) (#M6219) - suitable for DNA extraction from urine using automation

#### Formalin-Fixed Paraffin Embedded Tissue Samples

- Qiagen [QIAamp DNA FFPE Tissue Kit](#)
- Zymo Research [Quick-DNA™ FFPE Kit](#)

#### High Throughput Kits

- Qiagen [Gentra Puregene Blood Kit](#)
- Qiagen [Gentra Puregene Tissue Kit](#)
- Zymo Research [Quick-DNA™ Universal 96 Kit](#)

### RNA Extraction Kit

- Qiagen [AllPrep DNA/RNA Mini Kit](#)

### cDNA Synthesis

- ThermoFisher [SuperScript™ VILOTM cDNA Synthesis Kit](#)



## DNA QUANTIFICATION

DNA quantification should be performed in Lab Area 1. DNA should be stored at -20°C when not in use and at 4°C when in use. MassARRAY assays require pure DNA template with A260/A280 ratios between 1.7 – 2.0 and A260/A230 > 1.7. and a working dilution of at least 5-10 ng/µL.

It is recommended that the samples be checked for quality and quantity of amplifiable copies using Agena Bioscience's Sample ID Panel. Alternatively, you may use a fluorometric-based or spectrophotometric-based method for quantification. For oncology panels, fluorometric-based methods are recommended, because UV spectrophotometric-based methods will measure any nucleotides present in the sample, including RNA, dsDNA, ssDNA, and free nucleotides, which can give an inaccurate measurement of gDNA.

### [Agena's iPLEX® Pro Sample ID Panel](#)

Uniquely identify each sample using 44 SNPs and quantify the number of amplifiable copies of DNA template from as little as 500 to 18,000 copies (~1 ng to 60 ng) in a single reaction. The panel also provides gender identification.

### [Agena's Exome QC Panel](#)

Assess DNA identity, as well as quality and amplifiable template copy number, using 21 exonic SNPs, 3 markers for gender identification, and 25 competitive PCR assays in a single multiplexed reaction. The competitive PCR assays span a broad dynamic range of 100 – 100,000 copies (0.3 – 300 ng) and monitor sample fragment size over a 100 – 500 bp range.

### [Spectrophotometry](#)

[NanoDrop 8000](#) is a suitable UV-Vis spectrophotometer for DNA quantification.

### [Fluorometry](#)






[Qubit](#) and PicoGreen are suitable for DNA quantification.



## RECOMMENDED LAB EQUIPMENT AND CONSUMABLES

PRE-PCR EQUIPMENT				
Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
Plate Centrifuge (with appropriate rotors)	<a href="#">Eppendorf 5430/R</a>  <a href="#">Eppendorf 5810/R</a>		Max. RCF with plate rotor: 3,486 x g  <i>Substitutions acceptable</i>	All
Mini Tube Centrifuge	<a href="#">Eppendorf MiniSpin</a>		Recommend additional rotor option for 2 x 8-tube PCR strips.  <i>Substitutions acceptable</i>	All
Vortex	<a href="#">MO BIO Vortex Genie® 2 Vortex</a>		Variable speed; Suitable for tubes and plates.  <i>Substitutions acceptable</i>	All
Thermal Cycler	<a href="#">Veriti™ Thermal Cycler</a>  <a href="#">SimpliAmp™ Thermal Cycler</a>  <a href="#">ProFlex™ 96-well PCR System</a>  <a href="#">SensoQuest Labcycler Basic</a>		Choose appropriate block for 96/384.  <i>Substitutions acceptable</i>	All  Only needed in pre-PCR area for MassCLEAVE or cDNA synthesis
PCR Workstation with UV Irradiation	<a href="#">C.B.S. Scientific P-048-202</a>		UV light with timer; Dual UV bulb preferred. Min. 36" x 24" x 24" 48"x24"x24"  <i>Substitutions acceptable</i>	All
Mini Cooler	<a href="#">VWR 89511-788</a>		Optional For keeping enzymes cold while setting up reactions.  <i>Substitutions acceptable</i>	All



PRE-PCR EQUIPMENT				
Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
Refrigerator	<a href="#">VWR 14236-525</a>		Refrigerator for storing working reagents at 4 °C.  <i>Substitutions acceptable</i>	All
Freezer	<a href="#">VWR 97043-524</a>		Min. -20 °C for long-term DNA and reagent storage.  <i>Substitutions acceptable</i>	All
Electronic Multichannel Pipettes & Filtered Tips	<a href="#">Integra Voyager II 4731</a>  Alternate options: Manual multichannel pipettes may be used instead.		8- or 12-channel electronic adjustable tip spacing pipette; 0.5 µL – 12.5 µL  <i>Substitutions acceptable</i>	All
Manual Multichannel Pipettes & Filtered Tips	<a href="#">Rainin Pipet-Lite Multi Pipette XLS+</a>		8- or 12-channel pipette; 0.5 µL – 10 µL 20 µL - 200 µL  <i>Substitutions acceptable</i>	All
Single Channel Pipettes & Filtered Tips	<a href="#">Rainin Pipet-Lite LTS</a>  <a href="#">Eppendorf®</a>  <a href="#">Gilson</a>		Volume ranges: 0.1 µL – 2 µL 0.5 µL – 10 µL 10 µL – 100 µL 100 µL – 1000 µL  <i>Substitutions acceptable</i>	All



POST-PCR EQUIPMENT				
Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
Plate Centrifuge with appropriate plate rotor	<a href="#">Eppendorf 5430/R</a> <a href="#">Eppendorf 5810/R</a>		Max. RCF with plate rotor: 3,486 x g  <i>Substitutions acceptable</i>	All
Mini Tube Centrifuge	<a href="#">Eppendorf MiniSpin</a>		Recommend additional rotor option for 2 x 8-tube PCR strips.  <i>Substitutions acceptable</i>	All
Vortex	<a href="#">MO BIO Vortex Genie® 2 Vortex</a>		Variable speed; Suitable for tubes and plates.  <i>Substitutions acceptable</i>	All
Thermal Cycler	<a href="#">Veriti™ Thermal Cycler</a> <a href="#">SimpliAmp™ Thermal Cycler</a> <a href="#">ProFlex™ 96-well PCR System</a> <a href="#">SensoQuest Labcycler Basic</a>		Choose appropriate block for 96/384.  <i>Substitutions acceptable</i>	All
Plate/Tube Rotator	<a href="#">VWR 444-0502</a>		Tube rotator with 360° rotation. Use standard rotisserie to attach and rotate plates.  <i>Substitutions acceptable</i>	RS1000  UltraSEEK panels (all systems)
Mini Cooler	<a href="#">VWR 89511-788</a>		Optional For keeping enzymes cold while setting up reactions.  <i>Substitutions acceptable</i>	All






POST-PCR EQUIPMENT				
Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
Refrigerator	<a href="#">VWR 14236-525</a>		Refrigerator for storing working reagents at 4 °C.  <i>Substitutions acceptable</i>	All
Freezer	<a href="#">VWR 97043-524</a>		Min. -20 °C for long-term DNA and reagent storage.  <i>Substitutions acceptable</i>	All
Electronic Multichannel Pipettes & Filtered Tips	<a href="#">Integra Voyager II 4731</a>  Alternate option: Manual multichannel pipettes may be used instead.		8- or 12-channel electronic adjustable tip spacing pipette; 0.5 µL – 12.5 µL  <i>Substitutions acceptable</i>	All
Manual Multichannel Pipettes & Filtered Tips	<a href="#">Rainin Pipet-Lite Multi Pipette XLS+</a>		8- or 12-channel pipette; 0.5 µL – 10 µL 20 µL - 200 µL  <i>Substitutions acceptable</i>	All
Single Channel Pipettes & Filtered Tips	<a href="#">Rainin Pipet-Lite LTS</a>  <a href="#">Eppendorf®</a>  <a href="#">Gilson</a>		Volume ranges: 0.1 µL – 2 µL 0.5 µL – 10 µL 10 µL – 100 µL 100 µL – 1000 µL  <i>Substitutions acceptable</i>	All
Single Tube Magnetic Stand	<a href="#">V&amp;P Scientific VP-772F3</a>		Suitable for 1.5 mL microtubes with a single magnet.  <i>Substitutions acceptable</i>	UltraSEEK panels only





## POST-PCR EQUIPMENT

Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
96-well Plate Magnetic Stand	<a href="#">ThermoFisher DynaMag™-96 Side Magnet 12331DD</a>		Magnetic bead separation block for 96-well plates.  <i>Substitutions acceptable</i>	UltraSEEK panels only



CONSUMABLES				
Item	Manufacturer & Model	Image	Specifications/Notes	System Format
Microtubes	<a href="#">VWR 62111-756</a>		RNase-, DNase-, human DNA- and PCR inhibitor-free. Volume: 1.5 mL  <i>Substitutions acceptable</i>	All
PCR Strip Tubes	<a href="#">VWR 93001-118</a>		Optional 8- or 12-well strips with caps Volume: 0.2 mL  <i>Substitutions acceptable</i>	All
50 mL Tubes 15 mL Tubes 5 mL Tubes	<a href="#">VWR 82050-348</a>		For working aliquots of water. Sterile, RNase-, DNase- and human DNA-free.  <i>Substitutions acceptable</i>	All
Tube Racks	<a href="#">VWR Four-Way Microtube Racks</a>		<i>Substitutions acceptable</i>	All
Sealing Roller Tool & Paddle	<a href="#">VWR Excel Scientific Film Sealing Roller &amp; Paddles</a>		Paddles or roller for sealing plates  <i>Substitutions acceptable</i>	All
Reagent Reservoir	<a href="#">VWR 89094-662</a>		Disposable pipetting reservoirs; DNase- and RNase-free Volume: 25 mL; sterile (50 mL for UltraSEEK only)  <i>Substitutions acceptable</i>	All
Wash Bottles	<a href="#">VWR 10111-982</a>  <a href="#">414004-226</a>		For use with ethanol and water.  <i>Substitutions acceptable</i>	All



CONSUMABLES				
Item	Manufacturer & Model	Image	Specifications/Notes	System Format
Clear Adhesive Plate Seals	<a href="#">ThermoFisher Scientific AB0558</a>		Strong adhesive, -20 °C to 120 °C	All
Pressure Pads	<a href="#">Bio-Rad ADR5001</a>		Foam pad for uniform pressure on plates (for BioRad thermal cyclers)  <i>Substitutions acceptable</i>	All
96-well Microtiter Plate  <i>Verify compatibility with your thermal cyclers.</i>	Non-skirted: <a href="#">AB0600L</a> or equivalent  Semi-skirted: <a href="#">TF4483343</a> or equivalent	  	Non-skirted: Working vol.: 0.2 mL Max fill vol.: ≤0.3 mL  Semi-skirted, colored Working vol.: 0.2 mL Max. well vol.: 0.25 mL Allows for barcoding	96-format on the MassARRAY System with Chip Prep Module  For UltraSEEK, recommend semi-skirted, colored <b>only</b>
96-well Microtiter Plate <i>Verify compatibility with your thermal cyclers.</i>	Non-skirted: <a href="#">AB0600L</a> or equivalent		Non-skirted: Working vol.: 0.2 mL Max fill vol.: ≤0.3 mL	96-format on the MassARRAY System with RS1000 Nanodispenser
384-well Microtiter Plate <i>Verify compatibility with your thermal cyclers.</i>	<a href="#">TF0384</a> or equivalent		Full skirted: Working vol.: 25 µL Max vol.: 40 µL	384-format systems




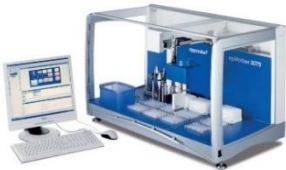
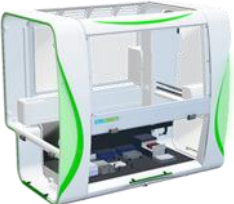


CONSUMABLES				
Item	Manufacturer & Model	Image	Specifications/Notes	System Format
HPLC-grade water	<a href="#">VWR JT4218-02</a>		Residue after evaporation $\leq 2$ ppm Sterile, nuclease- & DEPC-free. <i>Substitutions acceptable</i>	All
Ethanol	<a href="#">VWR 71006-012</a> Fisher Scientific <a href="#">BP2818500</a>		Absolute (200 proof), molecular biology grade. <i>Substitutions acceptable</i>	RS1000 (for cleaning tanks)
Tween 20	<a href="#">BioRad #1610781</a>  <a href="#">TFS 28320</a>		Nonionic detergent solution. 10% w/v Optional; for troubleshooting on the RS1000 Nanodispenser.	RS1000 only (for troubleshooting)
DNA AWAY™	<a href="#">MBP DNA AWAY™ 53509-506</a>		Eliminate DNase and DNA contamination  <i>Substitutions acceptable</i>	All
Type 1 Water	Milli-Q® <a href="#">Advantage A10 Water Purification System</a>		NCCLS, CAP or ATSM Type 1 water >18.2 M $\Omega$ Used for supply (rinse) water in CPM and RS 1000.  <i>Substitutions acceptable:</i> <i>DI water</i>	All
Isopropanol	<a href="#">EMD PX1835</a>		Molecular biology-grade	Chip Prep Module (for dispense tip cleaning)
NaOH	<a href="#">Honeywell Fluka 60-014-30</a>		0.1 M For pin conditioning on the RS1000 Nanodispenser.	RS1000 only




## OPTIONAL: AUTOMATED LIQUID HANDLING

The following equipment may be used for automating the sample extraction and pre- and post-PCR liquid handling steps. Agena Bioscience does not provide setup, configuration, methods, or support for this equipment.

Manufacturer & Model	Image	Specifications	System Format
<a href="#">Qiagen QIAcube</a>		Sample prep	All
<a href="#">Promega Maxwell® RSC</a>		DNA extraction and quantification from a wide range of sample types.	All
<a href="#">Hamilton Microlab NIMBUS</a>		Pre- or post-PCR liquid handling.  With enclosed UV light package for pre-PCR.	All
<a href="#">Eppendorf 5075</a>		Pre- or post-PCR liquid handling. Capable of handling streptavidin beads for UltraSEEK chemistry.	All
<a href="#">Perkin Elmer Janus</a>		Pre- or post-PCR liquid handling. Capable of handling streptavidin beads for UltraSEEK chemistry.	All



Manufacturer & Model	Image	Specifications	System Format
<a href="#">Biomek NX<sup>P</sup></a>		Pre- or post-PCR liquid handling. Capable of handling streptavidin beads for UltraSEEK chemistry. <a href="#">Biomek FX<sup>P</sup></a> recommended for high throughput automation.	All

## CUSTOMER SUPPORT

Please contact your local Agena Bioscience office for customer support.

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[0221]

**Revision History**

Revision	Date	Changes
R06	1/27/22	<ul style="list-style-type: none"> <li>Added DNA extraction kit recommended for the new Sample ID+ protocol.</li> <li>Added "or equivalent" to plate types required.</li> <li>Added SensoQuest Labcycler Basic as a thermal cycler option.</li> </ul>
R05	2/8/21	<ul style="list-style-type: none"> <li>In DNA Extraction section, changed Stratec to Invitex (PSP SalivaGene DNA Kit) due to company name change.</li> <li>For plate/tube rotator, corrected System Format to RS1000 and UltraSEEK Panels (Post-PCR Equipment section).</li> <li>Updated customer support contact information.</li> </ul>
R04	10/1/19	<ul style="list-style-type: none"> <li>Updated recommended microtiter plates.</li> <li>Added DI water as acceptable substitute for Type 1 water.</li> <li>Added NaOH (for pin conditioning on the RS1000 Nanodispenser).</li> <li>Added isopropanol (for pin cleaning on the Chip Prep Module, with Customer Support assistance).</li> <li>Clarified lab area activities.</li> <li>Updated checklist on page 2.</li> <li>Added SeraCare Reference Standards for somatic mutation positive controls.</li> <li>Updated recommended DNA extraction kits and added RNA extraction and cDNA synthesis kits.</li> <li>Updated DNA Quantification section, including addition of recommended fluorometers.</li> <li>Simplified thermal cycler recommendations.</li> <li>Added some additional options for some equipment and consumables.</li> </ul>

**Patents and Trademarks**

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The MassARRAY System, iPLEX Pro Sample ID Panel, Sample ID+, and iPLEX Pro Exome QC Panel are For Research Use Only. Not for use in diagnostic procedures.

1/27/22