



MassARRAY[®] 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[®] 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[®] Pro, iPLEX Gold, iPLEX HS, UltraSEEK[®], and MassCLEAVE[™] 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.
- Stratec [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).

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)	Eppendorf 5430/R Eppendorf 5810/R		Max. RCF with plate rotor: 3,486 x g <i>Substitutions acceptable</i>	All
Mini Tube Centrifuge	Eppendorf MiniSpin		Recommend additional rotor option for 2 x 8-tube PCR strips. <i>Substitutions acceptable</i>	All
Vortex	MO BIO Vortex Genie® 2 Vortex		Variable speed; Suitable for tubes and plates. <i>Substitutions acceptable</i>	All
Thermal Cycler	Veriti™ Thermal Cycler SimpliAmp™ Thermal Cycler ProFlex™ 96-well PCR System		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	C.B.S. Scientific P-048-202		UV light with timer; Dual UV bulb preferred. Min. 36" x 24" x 24" 48"x24"x24" <i>Substitutions acceptable</i>	All
Mini Cooler	VWR 89511-788		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	VWR 14236-525		Refrigerator for storing working reagents at 4 °C. <i>Substitutions acceptable</i>	All
Freezer	VWR 97043-524		Min. -20 °C for long-term DNA and reagent storage. <i>Substitutions acceptable</i>	All
Electronic Multichannel Pipettes & Filtered Tips	Integra Voyager II 4731 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	Rainin Pipet-Lite Multi Pipette XLS+		8- or 12-channel pipette; 0.5 µL – 10 µL 20 µL - 200 µL <i>Substitutions acceptable</i>	All
Single Channel Pipettes & Filtered Tips	Rainin Pipet-Lite LTS Eppendorf® Gilson		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	Eppendorf 5430/R Eppendorf 5810/R		Max. RCF with plate rotor: 3,486 x g <i>Substitutions acceptable</i>	All
Mini Tube Centrifuge	Eppendorf MiniSpin		Recommend additional rotor option for 2 x 8-tube PCR strips. <i>Substitutions acceptable</i>	All
Vortex	MO BIO Vortex Genie® 2 Vortex		Variable speed; Suitable for tubes and plates. <i>Substitutions acceptable</i>	All
Thermal Cycler	Veriti™ Thermal Cycler SimpliAmp™ Thermal Cycler ProFlex™ 96-well PCR System		Choose appropriate block for 96/384. <i>Substitutions acceptable</i>	All
Plate/Tube Rotator	VWR 444-0502		Tube rotator with 360° rotation. Use standard rotisserie to attach and rotate plates. <i>Substitutions acceptable</i>	All
Mini Cooler	VWR 89511-788		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	VWR 14236-525		Refrigerator for storing working reagents at 4 °C. <i>Substitutions acceptable</i>	All
Freezer	VWR 97043-524		Min. -20 °C for long-term DNA and reagent storage. <i>Substitutions acceptable</i>	All
Electronic Multichannel Pipettes & Filtered Tips	Integra Voyager II 4731 <u>Alternate option: Manual multichannel pipettes may be used instead.</u>		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	Rainin Pipet-Lite Multi Pipette XLS+		8- or 12-channel pipette; 0.5 µL – 10 µL 20 µL - 200 µL <i>Substitutions acceptable</i>	All
Single Channel Pipettes & Filtered Tips	Rainin Pipet-Lite LTS Eppendorf®, Gilson		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	V&P Scientific VP-772F3		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	ThermoFisher DynaMag™-96 Side Magnet 12331DD		Magnetic bead separation block for 96-well plates. <i>Substitutions acceptable</i>	UltraSEEK panels only






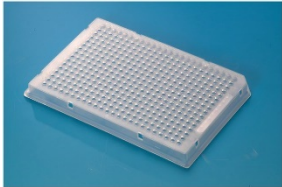


CONSUMABLES

Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
Microtubes	VWR 62111-756		RNase-, DNase-, human DNA- and PCR inhibitor-free. Volume: 1.5 mL <i>Substitutions acceptable</i>	All
PCR Strip Tubes	VWR 93001-118		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	VWR 82050-348	 15 5/10 50	For working aliquots of water. Sterile, RNase-, DNase- and human DNA-free. <i>Substitutions acceptable</i>	All
Tube Racks	VWR Four-Way Microtube Racks		<i>Substitutions acceptable</i>	All
Sealing Roller Tool & Paddle	VWR Excel Scientific Film Sealing Roller & Paddles		Paddles or roller for sealing plates <i>Substitutions acceptable</i>	All
Reagent Reservoir	VWR 89094-662		Disposable pipetting reservoirs; DNase- and RNase-free Volume: 25 mL; sterile (50 mL for UltraSEEK only) <i>Substitutions acceptable</i>	All
Wash Bottles	VWR 10111-982 414004-226		For use with ethanol and water. <i>Substitutions acceptable</i>	All



CONSUMABLES

Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
Clear Adhesive Plate Seals	ThermoFisher Scientific AB0558		Strong adhesive, -20 °C to 120 °C	All
Pressure Pads	Bio-Rad ADR5001		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: AB0600L Semi-skirted: TF4483343	 	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 only
96-well Microtiter Plate <i>Verify compatibility with your thermal cyclers.</i>	Non-skirted: AB0600L		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>	TF0384		Full skirted: Working vol.: 25 µL Max vol.: 40 µL	384-format systems







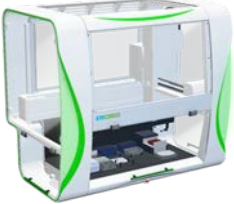
CONSUMABLES

Equipment	Manufacturer & Model	Image	Specifications/Notes	System Format
HPLC-grade water	VWR JT4218-02		Residue after evaporation ≤ 2 ppm Sterile, nuclease- & DEPC-free. <i>Substitutions acceptable</i>	All
Ethanol	VWR 71006-012 Fisher Scientific BP2818500		Absolute (200 proof), molecular biology grade. For cleaning tasks on the RS1000 Nanodispenser. <i>Substitutions acceptable</i>	RS1000 only
Tween 20	BioRad #1610781 TFS 28320		Nonionic detergent solution. 10% w/v Optional; for troubleshooting on the RS1000 Nanodispenser.	RS1000 only
DNA AWAY™	MBP DNA AWAY™ 53509-506		Eliminate DNase and DNA contamination <i>Substitutions acceptable</i>	All
Type 1 Water	Milli-Q® Advantage A10 Water Purification System		NCCLS, CAP or ATSM Type 1 water >18.2 M Ω Used for supply (rinse) water in CPM and RS 1000. <i>Substitutions acceptable: DI water</i>	All
Isopropanol	EMD PX1835		For pin cleaning on the Chip Prep Module, with Customer Support assistance.	Chip Prep Module only
NaOH	Honeywell Fluka 60-014-30		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
Qiagen QIAcube		Sample prep	All
Promega Maxwell® RSC		DNA extraction and quantification from a wide range of sample types.	All
Hamilton Microlab NIMBUS		Pre- or post-PCR liquid handling. With enclosed UV light package for pre-PCR.	All
Eppendorf 5075		Pre- or post-PCR liquid handling. Capable of handling streptavidin beads for UltraSEEK chemistry.	All
Perkin Elmer Janus		Pre- or post-PCR liquid handling. Capable of handling streptavidin beads for UltraSEEK chemistry.	All



Manufacturer & Model	Image	Specifications	System Format
Biomek NX^P		Pre- or post-PCR liquid handling. Capable of handling streptavidin beads for UltraSEEK chemistry. Biomek FX^P recommended for high throughput automation.	All

CUSTOMER SUPPORT

Please contact your local Agena Bioscience office for customer support.

AMERICAS

Help Desk: 1-877-4-GENOME or (+1) 858-882-9300

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[0419]



Revision History

Revision	Date	Changes
R04	10/1/19	<ul style="list-style-type: none">• Updated recommended microtiter plates.• Added DI water as acceptable substitute for Type 1 water.• Added NaOH (for pin conditioning on the RS1000 Nanodispenser).• Added isopropanol (for pin cleaning on the Chip Prep Module, with Customer Support assistance).• Clarified lab area activities.• Updated checklist on page 2.• Added SeraCare Reference Standards for somatic mutation positive controls.• Updated recommended DNA extraction kits and added RNA extraction and cDNA synthesis kits.• Updated DNA Quantification section, including addition of recommended fluorometers.• Simplified thermal cycler recommendations.• Added some additional options for some equipment and consumables.

Patents and Trademarks

MassARRAY, iPLEX, UltraSEEK, and Agena Bioscience are registered trademarks of Agena Bioscience, Inc. MassCLEAVE is a trademark of Agena Bioscience, Inc. All other trademarks or service marks set forth herein are the property of their respective owners.

Agena Bioscience's patented nucleic acid analysis by mass spectrometry methods and products are protected under United States patent rights including but not limited to; 6,440,705; 6,558,623; 6,730,517; 6,979,425; 6,994,969; 7,019,288; 7,025,933; 7,332,275; 7,390,672; 7,501,251; 7,888,127; 7,917,301; 8,003,317; 8,315,805; 8,349,566; 9,249,456; 9,310,378; 9,394,565; 9,669,376; and 9,896,724, and patents pending including but not limited to US20130017960, and foreign counterparts including but not limited to EP1173622B1, EP1727911B1, EP1546385B1, EP1332000B1, EP1613723B1, EP1660680B1, and EP2107129B1.

[0818]

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

10/1/19