

Axiom™ 2.0 Assay Mini 96-Array Format

SITE PREPARATION GUIDE

Manual Protocol

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Equipment and supplies required

This site preparation guide includes the supplier and part number information for the equipment, software, reagents, arrays, labware and other consumables that have been validated for use with the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol.

Information on suppliers of the equipment and supplies is listed in [Appendix A, "Supplier contact list" on page 13](#).

Thermo Fisher Scientific equipment, software, reagents, and arrays required

Table 1 Thermo Fisher Scientific equipment, software, reagents and arrays required

✓	Item	Part number
Equipment		
<input type="checkbox"/>	GeneTitan™ Multi-Channel Instrument ¹	Contact Thermo Fisher Scientific, Inc.
Software		
<input type="checkbox"/>	GeneChip™ Command Console™ (AGCC)	version 4.3 or later
<input type="checkbox"/>	Axiom™ Analysis Suite	version 1.1.1 or later
Reagents		
<input type="checkbox"/>	<p>Axiom™ 2.0 Assay Mini 96 Reagent Kit (1 required)</p> <p>Each Axiom 2.0 Assay Mini 96 Reagent Kit contains the following modules for processing two Mini 96-array format plates:</p> <ul style="list-style-type: none"> • Module 1 (qty 1) • Module 2-1 (qty 1) • Module 2-2 (qty 1) • Module 3 (qty 2 sets) <ul style="list-style-type: none"> – Axiom Wash Buffer A, 4 bottles – Axiom Wash Buffer B, 2 bottles – Axiom Water, 2 bottles • Module 4-1 (qty 1) • Module 4-2 (qty 1) • Axiom Hold Buffer (qty 1) 	<p>903013</p> <ul style="list-style-type: none"> • 901711 • 901528 • 901529 – 901446 – 901447 – 901578 • 901278 • 901276 • 903012

Table 1 Thermo Fisher Scientific equipment, software, reagents and arrays required (Continued)

✓	Item	Part number
Arrays		
<input type="checkbox"/>	Axiom™ myDesign™ Mini 96-Array Format Plates (2)	Varies, depending on array design
Consumable kits		
<input type="checkbox"/>	<p>Axiom 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (sufficient for processing 4 x Mini 96-array format plates)</p> <p>Each Kit Includes:</p> <ul style="list-style-type: none"> • 4 x Eppendorf 96 Deep-well Plate, 2000 µL • 60 x 25mL Matrix Reagent Reservoirs • 10 x Bio-Rad Hard Shell 96-well Plate • 4 x 96 Half-Skirt Plate 	902986
<input type="checkbox"/>	<p>Axiom™ 384HT High Volume Consumable Kit (sufficient for processing 5 x Mini 96-array format plates)</p> <p>Each Kit Includes:</p> <ul style="list-style-type: none"> • 10 x 384 Layout GeneTitan™ Stain Tray (Stain 1) • 5 x 384 Layout Axiom™ Stain2 Tray • 5 x 384 Layout Axiom™ Stab. Tray • 5 x 384 Layout Axiom™ Ligation Tray • 5 x 384 Layout GeneTitan™ Hyb Tray • 5 x 384 Layout GeneTitan™ Scan Tray • 25 x 384 Layout GeneTitan™ Scan and Stain Tray Cover <p>These trays are required for processing Axiom Mini 96-array format plates on the GeneTitan™ Multi-Channel Instrument. Please use the Axiom 384HT High Volume Consumable Kit when running the Axiom™ 2.0 Mini 96-Array Format Manual Protocol.</p>	902629
<input type="checkbox"/>	<p>Axiom™ Mini 96 Consumable Kit for QC</p> <p>Each kit includes:</p> <ul style="list-style-type: none"> • 10 x 96-well UV Plates 	903014
Training module		
<input type="checkbox"/>	<p>Axiom™ 2.0 Assay Mini 96 Training Kit</p> <p>Each Kit Includes:</p> <ul style="list-style-type: none"> • One Axiom™ 2.0 Assay Mini 96 Reagent Kit (2 x Mini 96 Reactions) • One Axiom™ Mini 96 Human AIMS Array Plate • One Axiom™ Mini 96 Manual Target Preparation Consumables Kit • One Axiom™ Mini 96 Consumable Kit for QC • One Axiom™ 384HT High Volume Consumable Kit • One full plate of 96 HapMap DNA samples 	<p>903015</p> <ul style="list-style-type: none"> • 903013 • 550414 • 902986 • 903014 • 902629 • 902451

¹ For a complete list of all equipment and supplies required for GeneTitan Instrument installation and operation, please consult the *GeneTitan™ Multi-Channel Instrument Site Preparation Guide* (P/N 08-0305).

Related documentation

- *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol User Guide*, P/N 703434
- *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol QRC*, P/N 703436
- *Axiom™ 2.0 gDNA Sample Preparation QRC*, P/N 702987
- *Axiom™ gDNA Sample Preparation for Axiom Genome-Wide BOS 1 Array Plate QRC*, P/N 702975
- *Axiom™ Genotyping Solution Data Analysis Guide*, P/N 702961
- *GeneTitan™ MC Protocol for Axiom 2.0 Array Plate Processing QRC*, P/N 702988
- *Applied Biosystems™ GeneChip™ Command Console™ 4.3 User Guide*, P/N 702569
- *Axiom™ Analysis Suite User Guide*, P/N 703307
- *GeneTitan™ Multi-Channel Instrument User Guide*, P/N 08-0308
- *GeneTitan™ Multi-Channel Instrument Site Preparation Guide*, P/N 08-0305
- *Axiom™ 384HT Array Plate Processing QRC*, P/N 703164

Equipment required for manual target preparation

Table 2 Additional instruments required for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

✓	Equipment	Manufacturer/ distributor	Part number
<input type="checkbox"/>	Two ovens (see "Oven requirements" on page 7)		
<input type="checkbox"/>	Fume hood (Strongly recommended: see the <i>Axiom 2.0 Assay Mini 96-Array-Format Manual Protocol User Guide</i> for more information)		
<input type="checkbox"/>	One of the following thermal cyclers: <ul style="list-style-type: none"> • Bio-Rad DNA Engine PTC-200/MJ PTC-200 Thermal Cycler • Bio-Rad / MJ Tetrad® 2 PTC-0240G • Applied Biosystems 9700 • Applied Biosystems 2720 (see "Thermal cycler recommendations" on page 8)	Bio-Rad Bio-Rad Thermo Fisher Scientific Thermo Fisher Scientific	
<input type="checkbox"/>	Plate shaker (see "Plate shaker recommendations" on page 9)		

Pre-amplification area/amplification staging area

Precautions are required when manipulating genomic DNA or setting up amplification reactions to avoid contamination with foreign DNA amplified in other reactions and procedures. It is recommended that genomic DNA manipulations and amplification reaction set up are performed in a dedicated rooms or areas separate from the main laboratory.

These areas should have dedicated sets of pipettes and plasticware. If no dedicated area is available, use of a dedicated bench or a dedicated biosafety hood and dedicated pipettes is suggested. If no dedicated bench or biosafety hood is available, a set of dedicated pipettes is recommended.

Ideally, the pre-amplification and amplification staging areas would be separate; however these areas may be combined due to space and equipment limitations.

Oven requirements

Multiple ovens are required for manual target preparation. The exact number depends whether you are running only a single sample plate and array plate through the workflow, or if you are running the three plate/week manual target preparation workflow.

- If you are running individual plates, you will need two ovens for the workflow.
- If you are running the three plate/week workflow, a third oven is highly recommended.

Refer to Chapter 6, “Manual Target Preparation for Processing Three Axiom Array Plates per Week” in the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol User Guide* (P/N 703434) for more information.

Table 3 Suggested settings for ovens when performing the three-plate workflow for manual target preparation

Day of workflow	Oven 1	Oven 2	Oven 3
Day 1	37°C	N/A	N/A
Day 2	37°C	65°C	37°C
Day 3	48°C ¹	65°C	37°C
Day 4	48°C ¹	65°C	37°C
Day 5	N/A	N/A	N/A

¹ For preheating of the 96-well metal chamber for hyb transfer.

Table 4 Ovens Required for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

✓	Equipment	Manufacturer	Part number
☐	Two ovens, any combination of the following types:	BINDER	ED056UL-120V Voltage: 120 V 1~60 Hz P/N 9010-0334
	• ED 56 Drying and Heating Chamber ¹		ED056-230V Voltage: 230 V 1~50/60 Hz P/N 9010-0333
	• GeneChip™ Hyb Oven 645 ²	Thermo Fisher Scientific	00-0331

¹ Replaces BINDER Model ED 53.

² The GeneChip™ Hybridization Oven 640 is currently not supported with the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol.

Spectrophotometer We recommend using one of the spectrophotometers listed in [Table 5](#).

Table 5 Spectrophotometers

✓	Item	Supplier	Part Number
☐	One of the following spectrophotometers: <ul style="list-style-type: none"> • DTX 880 Multimode Detector, with: ☐ Genomic Filter Slide or <ul style="list-style-type: none"> • SpectraMax® High throughput Microplate Spectrophotometer 	Beckman Coulter Beckman Coulter Molecular Devices	987921 – detector A30184 – filter slide Plus384

Thermal cycler recommendations

We have verified the performance of this assay using the following thermal cyclers:

- Bio-Rad DNA Engine PTC-200/MJ PTC-200, or
- Bio-Rad / MJ Tetrad® 2 PTC-0240G, or
- Applied Biosystems 9700 (with gold, silver, or aluminum block), or
- Applied Biosystems 2720

The performance of this assay has not been verified with other thermal cyclers. Use of other thermal cyclers may result in assay failure and may violate the Axiom Array and Reagent replacement policy. The thermal cycler needs to be programmed with the **Axiom 2.0 Denature** protocol:

1. 95°C 10 min
2. 48°C 3 min
3. 48°C hold

Use the heated lid option when setting up or running the protocol.

Thermal cycler consumables

[Table 6](#) provides details into the consumables to be used with each thermal cycler.

Table 6 Thermal cycler consumables for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Thermal cycler model	PCR plate type	Seal ¹
Bio-Rad PTC-200	<ul style="list-style-type: none"> • Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plate (Bio-Rad P/N HSP-9631) • Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad P/N HSS-9641)² 	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
Applied Biosystems 9700	<ul style="list-style-type: none"> • Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad P/N HSS-9641)² 	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
Applied Biosystems 2720	<ul style="list-style-type: none"> • Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad P/N HSS-9641)² 	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
Bio-Rad Tetrad® 2 PTC-0240	<ul style="list-style-type: none"> • Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plate (Bio-Rad P/N HSP-9631) • Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad P/N HSS-9641)² 	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)

¹ Microseal “B” film from Bio-Rad (P/N MSB-1001) may be used in place of MicroAmp Clear Adhesive Film for the Bio-Rad and Applied Biosystems thermal cyclers.

² Included in the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (P/N 902986)

Plate shaker recommendations

We recommend using one of the following shakers listed in [Table 7](#).

Table 7 Plate shakers

Shaker	Supplier	Part number
Thermo Scientific™ Compact Digital Microplate Shaker	Thermo Scientific	88880023
Jitterbug™	Boekel Scientific	Model 130 000

Plate centrifuge

One plate centrifuge is required for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol. We recommend the plate centrifuges listed in [Table 8](#). When centrifuging and drying pellets, the centrifuge must be able to spin down plates at:

- rcf: 3200 xg with an appropriate rotor—bucket combination (4000 RPM for the Eppendorf 5810R configuration described below)
- Temperature: 4°C

In addition, the bottom of the rotor buckets should be soft rubber to ensure that the deep well plates do not crack. Do not use buckets where the plates sit directly on a metal or hard plastic bottom. For the Eppendorf 5810R, do not use the A-4-62 rotor with a WO-15 plate carrier (hard bottom).

Table 8 Plate centrifuges recommended for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

✓	Item	Supplier	Part number
<input type="checkbox"/>	One of the following centrifuges:		
	<ul style="list-style-type: none"> • Allegra® 25R Refrigerated Benchtop Centrifuge 	Beckman Coulter	369434 (60 Hz, 280 V) 369435 (50/60 Hz, 200 V) 369436 (50 Hz, 230 V) 368954 S5700 Microplate rotor
	<ul style="list-style-type: none"> • Centrifuge 5810R (refrigerated) – A-4-81 MTP/Flex swinging bucket rotor with 4 microtest-plate buckets 	Eppendorf	022625501 (60 Hz, 120 V) 022625101 (50 Hz, 120 V, 20 AMP version) 022625551 (50 Hz, 230 V) 022638807
	<ul style="list-style-type: none"> • Sorvall® Legend® XTR (refrigerated), with: <input type="checkbox"/> TX-750 high visibility swinging rotor bucket <input type="checkbox"/> Carrier for microplates (including plate trays and neoprene pads) 	Thermo Scientific	75004521 (60 Hz, 120 V) 75004520 (50 Hz, 230 V) 75004523 (50/60 Hz, 230 V USA and Canada) 75003607 One of either: <ul style="list-style-type: none"> • Set of two carriers (75003795) • Set of four carriers (75003617)

Relative centrifugal force (*rcf*) can be calculated as follows:

$$rcf = (1.118 \times 10^{-5}) R S^2$$

where R is the radius of the rotor in centimeters, and S is the speed of the centrifuge in revolutions per minute.

Sample plates

Sample plates required for manual target prep are listed in [Table 9](#). These plates are available in the Axiom Mini 96 Manual Target Preparation Consumables Kit (P/N 902986) and the Axiom Mini 96 Consumable Kit for QC (P/N 903014), or purchased individually through the manufacturer or distributor.

Table 9 Sample plates required for Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

✓	Plate description	Manufacturer/ Distributor	Part number
<input type="checkbox"/>	Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate	Bio-Rad	HSS-9641
<input type="checkbox"/>	Eppendorf 96 Deep-well Plate, 2000 µL	Eppendorf	951033481
<input type="checkbox"/>	Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plate	Bio-Rad	HSP-9631
<input type="checkbox"/>	Greiner Bio-One 96 Well UV-Star® Microplate	E&K Scientific	25801

Pipettes and tips

Pipettes and tips recommended for manual target prep are listed in [Table 10](#).

Table 10 Recommended pipettes and tips for Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

✓	Equipment	Manufacturer/ distributor	Part number
<input type="checkbox"/>	Pipet-Lite™, Magnetic-Assist single channel P20	Rainin	L-20
<input type="checkbox"/>	Pipet-Lite™, Magnetic-Assist single channel P200	Rainin	L-200
<input type="checkbox"/>	Pipet-Lite™, Magnetic-Assist single channel P1000	Rainin	L-1000
<input type="checkbox"/>	Pipette, 12-channel P20	Rainin	L12-20
<input type="checkbox"/>	Pipette, 12-channel P50 (optional)	Rainin	L12-50
<input type="checkbox"/>	Pipette, 12-channel P200	Rainin	L12-200
<input type="checkbox"/>	Pipette, 12-channel P1200	Rainin	L12-1200
<input type="checkbox"/>	Pipette tips GP = refill	Rainin	GP-L10F
<input type="checkbox"/>	Pipette tips GP = refill	Rainin	GP-L200F
<input type="checkbox"/>	Pipette tips GP = refill	Rainin	GP-L1000F

Table 10 Recommended pipettes and tips for Axiom 2.0 Assay Mini 96-Array Format Manual Protocol (Continued)

✓	Equipment	Manufacturer/ distributor	Part number
<input type="checkbox"/>	Pipette tips RT = with rack	Rainin	RT-L10F
<input type="checkbox"/>	Pipette tips RT = with rack	Rainin	RT-L200F
<input type="checkbox"/>	Pipette tips RT = with rack	Rainin	RT-L1000F

Other labware
required

Table 11 Other labware required for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

✓	Item	Manufacturer/ distributor	Part number
<input type="checkbox"/>	Microcentrifuge tube holder	various	various
<input type="checkbox"/>	96-well PCR Plate Rack	various	various
<input type="checkbox"/>	Conical tube, 50 mL	various	various
<input type="checkbox"/>	Conical tube, 15 mL	various	various
<input type="checkbox"/>	Tube Holder, 15 and 50 mL	various	various
<input type="checkbox"/>	Matrix™ 25 mL reservoir	Thermo Scientific	8093-11
<input type="checkbox"/>	96 well plate metal chamber	Diversified Biotech	CHAM-1000
<input type="checkbox"/>	Serological pipettes (10 mL)	VWR	89130-898
<input type="checkbox"/>	Serological pipettes (5 mL)	VWR	89130-896
<input type="checkbox"/>	Adhesive film for 96-well plates – use one of the following: • MicroAmp® Clear Adhesive Film	Applied Biosystems	4306311
	• Microseal 'B' Film	Bio-Rad	MSB1001
<input type="checkbox"/>	Kimwipes®	Your choice	—

Other reagents and
gels required

Table 12 Other reagents required

✓	Item	Supplier	Part number
<input type="checkbox"/>	2-Propanol, anhydrous, 99.5% (Isopropanol)	Sigma-Aldrich	278475
<input type="checkbox"/>	Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Thermo Fisher Scientific	75793

Table 13 Reagents and gels required to run sample QC steps

✓	Item	Supplier	Part number
<input type="checkbox"/>	Mother E-Base™ Device	Thermo Fisher Scientific (formerly Life Technologies™ /Invitrogen)	EB-M03
<input type="checkbox"/>	Daughter E-Base™ Device		EB-D03
<input type="checkbox"/>	E-Gel® 48 4% agarose gels (for Sample QC)		G8008-04
<input type="checkbox"/>	TrackIt™ 25 bp DNA Ladder (for Sample QC)		10488-022
<input type="checkbox"/>	TrackIt™ Cyan/Orange Loading Buffer (for Sample QC)		10482-028
<input type="checkbox"/>	E-Gel® 48 1% agarose gels (for gDNA QC)		G8008-01
<input type="checkbox"/>	RediLoad™ (for gDNA QC)		750026
<input type="checkbox"/>	E-Gel® 96 High Range DNA Marker (for gDNA QC)		12352-019
<input type="checkbox"/>	Water, nuclease-free, ultrapure MB grade		71786

Other common lab equipment required

The remaining equipment required is listed in [Table 14](#).

Table 14 Other common lab equipment required

✓	Item	Supplier	Part number
<input type="checkbox"/>	Freezer, -20°C	Any vendor	—
<input type="checkbox"/>	Refrigerator, 2 to 8°C	Any vendor	—
<input type="checkbox"/>	Vortex-Genie® (for plates and microtubes) ¹	Scientific Industries	SI-0236 (120 V/60 Hz) SI-0246 (230 V/50 Hz)
<input type="checkbox"/>	Mini Microcentrifuge, for 2 mL tubes*	VWR	93000-196 (120V) 93000-196 (230V)
<input type="checkbox"/>	Bel-Art Cryo-Safe Mini Cooler, -15°C ¹	VWR	47751-730
<input type="checkbox"/>	Ice bucket, 4 to 9 liters	Any vendor	—
<input type="checkbox"/>	Markers, permanent, fine point	Your choice	—
<input type="checkbox"/>	Pipet-Aid*	VWR	53106-220

¹ Equivalent items from other manufacturers are acceptable.



Supplier contact list

Table 1 Supplier contact list

Supplier	Website address
Applied Biosystems	www.appliedbiosystems.com
Beckman Coulter	www.beckmancoulter.com
Bio-Rad	www.bio-rad.com
E&K Scientific	www.eandkscientific.com
Eppendorf	www.eppendorf.com
Rainin	www.shoprainin.com
Scientific Industries	www.scientificindustries.com
Sigma-Aldrich	www.sigmaaldrich.com
Thermo Fisher Scientific (Thermo Scientific, Applied Biosystems, Invitrogen, Fisher Scientific, Affymetrix)	www.thermofisher.com
USB Corporation (Thermo Fisher Scientific)	www.usb.affymetrix.com
VWR	www.vwr.com

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