Affymetrix®

Site Preparation Guide

Axiom® 2.0 Assay Manual Workflow

P/N 702991 Rev. 3

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Equipment and Supplies for the Axiom® 2.0 Assay Manual Workflow

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 Affymetrix® Axiom® 2.0 Assay Manual Workflow.

Information on suppliers of the equipment and supples is listed in Appendix A, *Supplier Contact List* on page 12.

Affymetrix Equipment, Software, Reagents and Arrays Required

Table 1.1 Affymetrix Equipment, Software, Reagents and Arrays Required

✓	Item	Part Number
Equipment		
❑	GeneTitan® Multi-Channel Instrument*	Contact Affymetrix, Inc.
Software		
❑	GeneChip® Command Console® (AGCC)	version 3.1 or later
❑	Genotyping Console	version 4.1 or later
Reagents		
❑	Axiom® 2.0 Reagent Kit	901758 (96 reactions)
Arrays		
Axiom® Genotyping Solution Array Plates:		
❑	Please visit www.affymetrix.com for a complete list of array plates available for use with the Axiom® 2.0 Assay.	
Consumables		
❑	Each Axiom® GeneTitan® Consumables Kit contains the following labware for each array plate: ▫ 1 Hyb Tray ▫ 1 Scan Tray with top cover and protective base ▫ 5 Stain Trays with top covers These trays are required for processing Axiom array plates on the GeneTitan® Multichannel Instrument. NOTE: Consumables are included with the Axiom Genome-Wide BOS and Pan-African Genotyping Bundles.	901606

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

Table 1.1 Affymetrix Equipment, Software, Reagents and Arrays Required (Continued)

✓	Item	Part Number
Training Module		
❑	Axiom® 2.0 Assay Training Module for CEU Arrays	901767
❑	Axiom® 2.0 Assay Training Module for ASI Arrays	901768
❑	Axiom® 2.0 Assay Training Module for CHB Arrays	901770
❑	Axiom® 2.0 Assay Training Module for EUR Arrays	901771
❑	Axiom® 2.0 Assay Training Module for BOS Arrays Each Kit Includes: ▪ Two Genome-Wide 96-Array Plates for specific array plate types listed above, except for BOS which includes 1 CEU Array Plate and 1 BOS Array Plate. ▪ Two Axiom® 2.0 Reagent Kits (96 reactions) ▪ One full plate of 96 HapMap DNA samples ▪ One half plate of 48 HapMap DNA samples* (Not supplied in the non-human training modules) ▪ One quarter plate of 24 HapMap DNA samples (Not supplied in the non-human training modules) ▪ One Zerostat Anti-Static Gun	901750
❑	Axiom® 2.0 Pan-African Training Module Each Kit Includes: ▪ One Axiom Genome-Wide Pan-African Genotyping Bundle (1 set of 96-array plates, 3 Axiom 2.0 Reagent Kits, and 3 Axiom GeneTitan Consumables Kits) ▪ Three identical 96 HapMap DNA plates ▪ One Zerostat Anti-Static Gun	901769

** For non-human Axiom arrays, you must provide your own positive control that meets the genomic DNA requirements specified by the Array Plates and Reagent Replacement Policy.*

Related Affymetrix Documentation

- *Axiom® 2.0 Assay Manual Workflow User Guide*, P/N 702990
- *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991
- *Axiom® 2.0 Assay Manual Target Prep Protocol QRC*, P/N 702989
- *Axiom® 2.0 gDNA Sample Prep Protocol QRC*, P/N 702987
- *Axiom® gDNA Sample Prep for Axiom Genome-Wide BOS 1 Array Plate QRC*, P/N 702975
- *Axiom® Genotyping Solution Analysis Guide*, P/N 702961
- *GeneTitan® MC Protocol for Axiom 2.0 Array Plate Processing QRC*, P/N 702988
- *Affymetrix® GeneChip® Command Console® 3.1 User Manual*, P/N 702569
- *Affymetrix® Genotyping Console™ 4.1 User Manual*, P/N 702982
- *Axiom® 2.0 Assay Automated Workflow User Guide*, P/N 702963
- *Axiom® 2.0 Assay Automated Target Prep Protocol QRC*, P/N 702962
- *Affymetrix® GeneTitan® MultiChannel Instrument User's Guide*, P/N 08-0306
- *Affymetrix® GeneTitan® MultiChannel Instrument Site Preparation Guide*, P/N 08-0305

Equipment Required for Manual Target Prep

Table 1.2 Additional Instruments Required for the Axiom® 2.0 Assay Manual Workflow

P	Equipment	Manufacturer/ Distributor	Part Number
❑	Two or three ovens (see *Oven Requirements* on page 7)		
❑	Fume Hood (Strongly recommended: see the *Axiom 2.0 Assay Manual Workflow User Guide* for more information)		
❑	One of the following Thermal Cyclers: ▪ Bio-Rad DNA Engine PTC-200/MJ PTC-200 Thermal Cycler ▪ Whatman Biometra TRobot 96 ▪ Bio-Rad / MJ Tetrad® 2 PTC-0240G ▪ ABI 9700 ▪ ABI 2720 (see *Thermal Cycler Recommendations*, below)	 Bio-Rad Biometra Bio-Rad ABI ABI	
❑	One of the following shakers:		
	Titer Plate Shaker- 4PL, 120V	Thermoscientific	4625
	or		
	Jitterbug™	Boekel Scientific	Model 130 000

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 upon whether you are running only a single sample plate and array plate through the workflow, or if you are trying to run 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 the chapter, *Processing Three Axiom Array Plates per Week with Manual Target Preparation* in the *Axiom® 2.0 Assay Manual Workflow User Guide* (P/N 702990) for more information.

Table 1.3 Suggested Settings for Ovens When Performing Three Plate/week Manual Target Prep Workflow

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	65°C	37°C
Day 4	48°C	65°C	37°C
Day 5	N/A	N/A	N/A

Table 1.4 Ovens Required for the Axiom® 2.0 Assay Manual Workflow

✓	Equipment	Manufacturer/ Distributor	Part Number
❑	Two to three ovens, any combination of the following types:		
	▪ ED 53 drying oven by Binder	VWR	50/60 Hz, 115 V, 1200W VWR P/N 47746-744 Mfg. P/N 9010-0131
			50/60 Hz, 230 V, 1200 W VWR P/N 47746-690 Mfg. P/N 9010-0078
	▪ Affymetrix® GeneChip® Hyb Oven 645*	Affymetrix	00-0331

** The GeneChip® Hybridization Oven 640 is currently not supported with the Axiom 2.0 Assay; however, if you want to utilize it in the workflow please contact your Field Service Engineer (FSE) or Affymetrix Technical Support regarding the compatibility of this oven with the Axiom 2.0 Assay.*

Spectrophotometer

We recommend using one of the spectrophotometers listed in Table 1.5.

Table 1.5 Spectrophotometers

✓	Item	Supplier	Part Number
❑	One of the following spectrophotometers:		
	▪ DTX 880 Multimode Detector, with:	Beckman Coulter	987921 – detector
	❑ Genomic Filter Slide	Beckman Coulter	A30184 – filter slide
	or		
	▪ SpectraMax High throughput Microplate Spectrophotometer	Molecular Devices	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
- Whatman Biometra TRobot 96, or
- Bio-Rad / MJ Tetrad® 2 PTC-0240G, or
- ABI 9700 (with gold, sliver, or aluminum block), or
- ABI 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 1.6 provides details into the consumables to be used with each thermal cycler.

Table 1.6 Thermal Cycler Consumables for the Axiom 2.0 Assay Manual Workflow

Thermal Cycler Model	PCR Plate Type	Seal*
Bio-Rad PTC-200	Bio-Rad Hard-Shell Thin-Wall 96-Well Skirted PCR Plates, P/N HSP-9631	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
TRobot	Bio-Rad Hard-Shell Thin-Wall 96-Well Skirted PCR Plates, P/N HSP-9631	Bio-Rad Arched Auto-Sealing Lids with Wide Tabs (p/n MSL-2032) with Bio-Rad Micro seal 'P' Replacement Pads (MSP-1003)
Bio-Rad Tetrad 2 PTC-0240	Bio-Rad Hard-Shell Thin-Wall 96-Well Skirted PCR Plates, P/N HSP-9631	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
ABI 9700	Bio-Rad P/N HSS-9601 (half skirted plate)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
ABI 2720	Bio-Rad P/N HSS-9601 (half skirted plate)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)

** 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 ABI thermal cyclers.*

Plate Centrifuge

One plate centrifuge is required for the Axiom® 2.0 Assay Manual Workflow. We recommend the plate centrifuges listed in Table 1.7. When centrifuging and drying pellets, the centrifuge must be able to spin down plates at:

- rcf: 3200 x g 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 1.7 Plate Centrifuges Recommended for the Axiom® 2.0 Assay Manual Workflow

✓	Item	Supplier	Part Number
❑	One of the following centrifuges:		
	▪ 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
	▪ Centrifuge 5810R (refrigerated)	Eppendorf	022625501 (60 Hz, 120 V) 022625101 (50 Hz, 120 V, 20 AMP version) 022625551 (50 Hz, 230 V)
	❑ A-4-81 MTP/Flex swinging bucket rotor with 4 microtest-plate buckets		022638807
	▪ Sorvall® Legend® XTR (refrigerated), with:	Thermo Scientific	75004521 (60 Hz, 120 V) 75004520 (50 Hz, 230 V) 75004523 (50/60 Hz, 230 V USA and Canada)
	❑ TX-750 high visibility swinging rotor bucket		75003607
	❑ Carrier for microplates (including plate trays and neoprene pads)		One of either: ▪ 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.

Plates

Table 1.8 Plates Required for Axiom 2.0 Assay Manual Workflow

✓	Equipment	Manufacturer/ Distributor	Part Number
❑	ABgene® 96 Square Well Storage Plate Mark II, 2.2 mL	Thermo Scientific	AB-0932
❑	Bio-Rad Hard Shell 96-well plate	Bio-Rad	HSP-9631
❑	96-well UV Star Plates, 370 µL/well	E&K Scientific	25801
❑	Hard-Shell Full-Height 96-Well Semi- Skirted PCR Plate (required only if using ABI 2720 or ABI 9700 Thermal Cyclers)	Bio-Rad	HSS-9601

Pipettes and Tips

Pipettes and tips recommended for Manual target prep are listed in Table 1.9.

Table 1.9 Recommended Pipettes and Tips for Axiom 2.0 Assay Manual Workflow

✓	Equipment	Manufacturer/ Distributor	Part Number
❑	Pipet-Lite™, Magnetic-Assist single channel P20	Rainin	L-20
❑	Pipet-Lite™, Magnetic-Assist single channel P200	Rainin	L-200
❑	Pipet-Lite™, Magnetic-Assist single channel P1000	Rainin	L-1000
❑	Pipette, 12-channel P20	Rainin	L12-20
❑	Pipette, 12-channel P50 (optional)	Rainin	L12-50
❑	Pipette, 12-channel P200	Rainin	L12-200
❑	Pipette, 12-channel P1200	Rainin	L12-1200
❑	Pipette tips GP = refill	Rainin	GP-L10F
❑	Pipette tips GP = refill	Rainin	GP-L200F
❑	Pipette tips GP = refill	Rainin	GP-L1000F
❑	Pipette tips RT = with rack	Rainin	RT-L10F
❑	Pipette tips RT = with rack	Rainin	RT-L200F
❑	Pipette tips RT = with rack	Rainin	RT-L1000F

Other Labware Required

Table 1.10 Other Labware Required for the Axiom 2.0 Assay Manual Workflow

✓	Item	Manufacturer/ Distributer	Part Number
❑	Conical tube, 50 mL	VWR	21008-178
❑	Conical tube, 15 mL	VWR	21008-202
❑	Tube Holder, Falcon, 50 mL	various	various
❑	Solution Basin, 100 mL sterile, multichannel	VWR	89092-836
❑	96 well plate metal chamber	Diversified Biotech	CHAM-1000
❑	Serological Pipettes (10 mL)	VWR	89130-898
❑	Serological Pipettes (5 mL)	VWR	89130-896
❑	Adhesive film for 96-well plates – use one of the following:		
	▪ MicroAmp® Clear Adhesive Film	Applied Biosystems	4306311
	▪ Microseal 'B' Film	Bio-Rad	MSB1001
❑	Kimwipes®	Your choice	—

Other Reagents and Gels Required

Table 1.11 Other Reagents Required

✓	Item	Supplier	Part Number
❑	2-Propanol, anhydrous, 99.5% (Isopropanol)	Sigma-Aldrich	278475
❑	Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Affymetrix	75793

Table 1.12 Reagents and Gels Required to Run QC Steps

✓	Item	Supplier	Part Number
❑	Mother E-Base™ Device	Life Technologies™ (formerly Invitrogen)	EB-M03
❑	Daughter E-Base™ Device		EB-D03
❑	E-Gel® *48* 4% agarose gels (for Axiom QC)		G8008-04
❑	TrackIt™ 25 bp DNA Ladder (for Axiom QC)		10488-022
❑	TrackIt™ Cyan/Orange Loading Buffer (for Axiom QC)		10482-028
❑	E-Gel® *48* 1% agarose gels (for gDNA QC)		G8008-01
❑	RediLoad™ (for gDNA QC)		750026
❑	E-Gel® *96* High Range DNA Marker (for gDNA QC)		12352-019
❑	Water, nuclease-free, ultrapure MB grade (only required for performing rehybridization protocols and gel QC)	Affymetrix	71786

Other Lab Equipment Required

The remaining equipment required is listed in Table 1.13.

Table 1.13 Other Equipment Required

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

** Equivalent items from other manufacturers are acceptable.*

Supplier Contact List

Table A.1 Supplier Contact List

Supplier	Web Site Address
Affymetrix	www.affymetrix.com
Applied Biosystems	www.appliedbiosystems.com
Beckman Coulter	www.beckmancoulter.com
Bio-Rad	bio-rad.com
E&K Scientific	eandkscientific.com
Eppendorf	eppendorf.com
Life Technologies (Invitrogen)	www.lifetechnologies.com
Rainin	www.rainin.com
Scientific Industries	www.scientificindustries.com
Sigma-Aldrich	www.sigmaaldrich.com
TEKnova	www.teknova.com
Thermo Scientific	www.thermofisher.com
USB Corporation (Affymetrix)	www.usb.affymetrix.com
VWR	vwr.com
Biometra	www.biometra.com