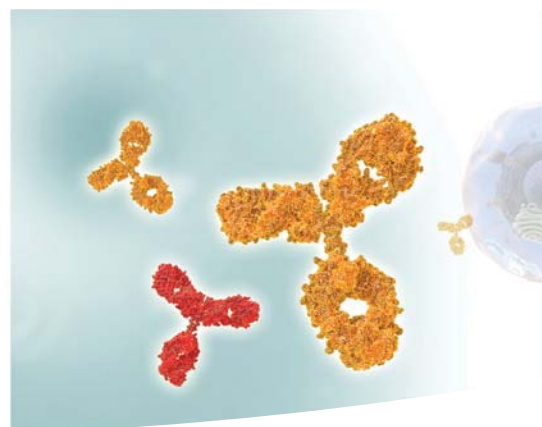
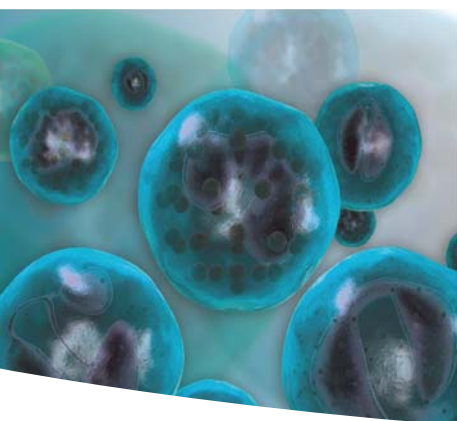


PharmacoScan™ Assay 24-Array Format

Manual Protocol

SitePreparationGuide



eBioscience

GeneChip

USB

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Equipment and Supplies for the PharmacoScan™ Assay 24-Array Format Manual Protocol

Information on suppliers of the equipment and supplies is listed in [Appendix A, *Supplier Contact List*](#) on [page 12](#).

Affymetrix Equipment, Software, Reagents and Arrays Required

✓	Item	Part Number
Equipment		
❑	GeneTitan® Multi-Channel Instrument*	Contact Affymetrix, Inc.
Software		
❑	GeneChip® Command Console® (AGCC)	version 4.3 or later
❑	Axiom™ Analysis Suite	version 2.0 or later
Reagents		
❑	PharmacoScan™ Reagent Kit 4x24 Reactions (1 required) Each PharmacoScan Reagent Kit 4x24 Reactions contains the following modules for processing four 24-array format plates:	902908
	■ PharmacoScan Module A (qty 1, contains 4 vials)	■ 902896
	■ PharmacoScan Module B (qty 1, contains 4 vials)	■ 902897
	■ Module 1 (qty 1)	■ 901711
	■ Module 2-1 (qty 1)	■ 901528
	■ Module 2-2 (qty 1)	■ 901529
	■ Module 3 (qty 4)	
	❑ Wash Buffer A	❑ 901446
	❑ Wash Buffer B	❑ 901447
	❑ Water	❑ 901578
	■ Module 4-1 (qty 1)	■ 901278
	■ Module 4-2 (qty 1)	■ 901276
	■ Module 5-1 (qty 1)	■ 902796
	■ Module 5-2 (qty 1)	■ 902797
Arrays		
❑	PharmacoScan™ 24F Array Plates (4)	902994
PharmacoScan™ Array GeneTitan Consumables		
❑	Each Axiom® GeneTitan® Consumables Kit contains the following labware for each array plate:	901606
	■ 1 Hyb Tray	
	■ 1 Scan Tray with top cover and protective base	
	■ 5 Stain Trays with top covers	
	These trays are required for processing PharmacoScan array plates on the GeneTitan® Multi-Channel Instrument.	

* 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).

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

✓	Item	Part Number
PharmacoScan™ Kits		
☐	PharmacoScan™ Training 24F Kit for Manual Protocol Each Kit Includes: <ul style="list-style-type: none"> ■ PharmacoScan™ Reagent Kit 4x24 Reactions ■ Four PharmacoScan™ 24F Array Plates ■ Four Axiom® GeneTitan® Consumables Kits ■ Two PharmacoScan™ DNA Training Plates, for 24 format Assay 	903011
☐	PharmacoScan™ Assay 24F Kit for Manual Protocol Each Kit Includes: <ul style="list-style-type: none"> ■ PharmacoScan™ Reagent Kit 4x24 Reactions ■ Four PharmacoScan™ 24F Array Plates ■ Four Axiom® GeneTitan® Consumables Kits 	903010

Related Documentation

- *PharmacoScan™ Assay 24-Array Format Manual Protocol User Guide*, P/N 703286
- *PharmacoScan™ Assay 24-Array Format Manual Protocol QRC*, P/N 703288
- *Axiom™ Analysis Suite User Guide*, P/N 703307
- *Axiom® Genotyping Solution Data Analysis Guide*, P/N 702961
- *GeneTitan® MC Protocol for Axiom 2.0 Array Plate Processing QRC*, P/N 702988
- *Affymetrix® GeneChip® Command Console® 4.0 User Guide*, P/N 702569
- *Affymetrix® Genotyping Console™ 4.2 User Guide*, P/N 702982
- *GeneTitan® Multi-Channel Instrument User Guide*, P/N 08-0308
- *GeneTitan® Multi-Channel Instrument Site Preparation Guide*, P/N 08-0305

Equipment Required for Manual Target Prep

Table 1.2 Additional Instruments Required for the PharmacoScan™ Assay 24-Array Format Manual Protocol

✓	Equipment	Manufacturer/ Distributor	Part Number
☐	Two ovens (see Oven Requirements on page 6)		
☐	Fume Hood (Strongly recommended: see the <i>PharmacoScan™ Assay 24-Array Format Manual Protocol User Guide</i> for more information)		
☐	Thermal Cycler: <ul style="list-style-type: none"> ■ ABI 9700 ■ ABI Veriti™ ■ ABI ProFlex™ ■ Eppendorf Mastercycler™ pro S (see Thermal Cycler Recommendations and Protocols on page 7)	ABI ABI ABI Eppendorf	
☐	One of the following shakers: <ul style="list-style-type: none"> ■ Compact Digital Microplate Shaker ■ Jitterbug™ 	Thermo Scientific Boekel Scientific	88880023 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

Two ovens are required for PharmacoScan manual target preparation.

Table 1.3 Ovens Required for the PharmacoScan Assay 24-Array Format Manual Protocol

✓	Equipment	Manufacturer	Part Number
□	Two ovens, any combination of the following types:		
	■ ED 56 Drying and Heating Chamber*	BINDER	ED056UL-120V Voltage: 120 V 1~60 Hz P/N 9010-0334
			ED056-230V Voltage: 230 V 1~50/60 Hz P/N 9010-0333
	■ Affymetrix® GeneChip® Hyb Oven 645†	Affymetrix	00-0331

* Replaces BINDER Model ED 53.

† The GeneChip® Hybridization Oven 640 is currently not supported with the PharmacoScan Assay 24-Array Format Manual Protocol.

Spectrophotometer

We recommend using one of the spectrophotometers listed in [Table 1.4](#).

Table 1.4 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 and Protocols

The following thermal cycler has been validated for the PharmacoScan Assay 24-Array Format Manual Protocol:

- ABI 9700 (with gold-plated or silver block)
- ABI Veriti
- ABI ProFlex
- Eppendorf Mastercycler pro S

! IMPORTANT: Always use the heated lid option when programming protocols. The PharmacoScan mPCR protocol was validated using the “9600 mode” on the ABI 9700, ABI Veriti, and ABI ProFlex thermal cyclers. The “Safe” mode was used for the Eppendorf Mastercycler pro S. Refer to the manufacturer’s user guide for programming information.

Figure 1.1 PharmacoScan mPCR Thermal Cycler Protocol (Stage 1A)

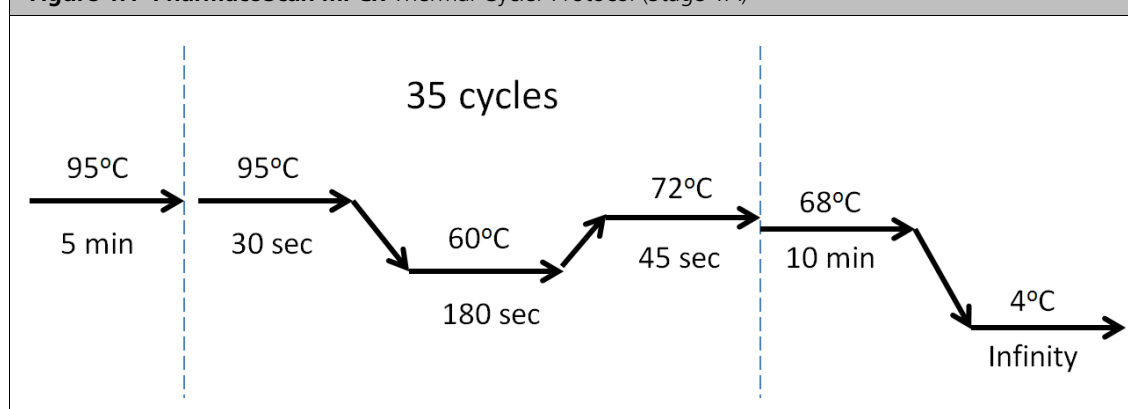
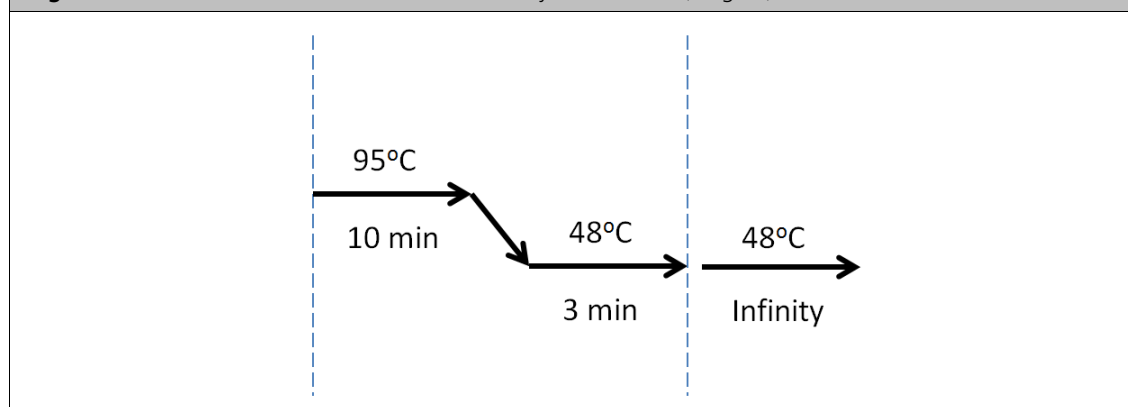


Figure 1.2 PharmacoScan Denature Thermal Cycler Protocol (Stage 4)



▲ WARNING: Evaporation during denaturation can negatively impact assay performance. Use the recommended thermal cycler consumables and sealing film to eliminate condensation and evaporation.

PharmacoScan Assay 24-Array Format Manual Protocol has been validated with the ABI 9700 (with gold-plated or silver block) ABI Veriti, ABI ProFlex, and Eppendorf Mastercycler pro S. Use of other thermal cyclers for this stage may result in assay failure and may violate the array and reagent replacement policy.

Thermal Cycler Consumables

Table 1.5 provides details into the consumables to be used with each thermal cycler.

Table 1.5 Thermal Cycler Consumables for the PharmacoScan Assay 24-Array Format Manual Protocol

Thermal Cycler Model	PCR Plate Type	Seal*
ABI 9700	■ BioRad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (P/N HSS-9641)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
ABI Veriti	■ BioRad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (P/N HSS-9641)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
ABI ProFlex	■ BioRad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (P/N HSS-9641)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
Eppendorf Mastercycler pro S	■ BioRad Hard-Shell Low Profile 96-well Full-Skirt PCR Plate (P/N HSP-9631)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)

*Microseal 'B' film from BioRad (P/N MSB-1001) may be used in place of MicroAmp Clear Adhesive Film for the ABI thermal cycler.

Plate Centrifuge

One plate centrifuge is required for the PharmacoScan Assay 24-Array Format Manual Protocol. We recommend the plate centrifuges listed in Table 1.6. 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 1.6 Plate Centrifuges Recommended for the PharmacoScan Assay 24-Array Format Manual Protocol

✓	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.

Sample Plates

Table 1.7 Sample Plates Required for PharmacoScan Assay 24-Array Format Manual Protocol

✓	Plate Description	Manufacturer/ Distributor	Part Number
❑	One of the following PCR Plates: <ul style="list-style-type: none"> ■ BioRad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (if using ABI 9700, ABI Veriti, ABI ProFlex thermal cyclers) ■ BioRad Hard-Shell Low Profile 96-well Full-Skirt PCR Plate (if using Eppendorf Master pro S thermal cyclers) 	Bio-Rad	HSS-9641
❑	ABgene® 96 Square Well Storage Plate Mark II, 2.2 mL	Thermo Scientific	AB-0932
❑	96-well UV Star Plates, 370 µL/well	E&K Scientific VWR, Sigma-Aldrich	25801 655801

Pipettes and Tips

Pipettes and tips recommended for PharmacoScan target preparation are listed in [Table 1.8](#).

Table 1.8 Recommended Pipettes and Tips for PharmacoScan Assay 24-Array Format Manual Protocol

✓	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 1.9 Other Labware Required for the PharmacoScan Assay 24-Array Format Manual Protocol

✓	Item	Manufacturer/ Distributor	Part Number
❑	Microcentrifuge tubes and tube holder	various	various
❑	8 well strip tubes with caps	various	various
❑	50 mL conical centrifuge tube, polypropylene, sterile	various	various
❑	15 mL conical centrifuge tube, polypropylene, sterile	various	various
❑	Tube Holder, 15 and 50 mL	various	various
❑	Matrix™ 25 mL divided reservoir	Thermo Fisher Scientific	8095
❑	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 ■ Microseal 'B' Film	Applied Biosystems Bio-Rad	4306311 MSB1001
❑	Kimwipes®	Your choice	—

Other Reagents and Gels Required

Table 1.10 Other Reagents Required

✓	Item	Supplier	Part Number
❑	QIAGEN Multiplex PCR <i>Plus</i> Kit (100)	QIAGEN	206152
❑	Quant-iT PicoGreen dsDNA Assay Kit (recommended for DNA quantitation)	Thermo Fisher Scientific	P7589
❑	Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Affymetrix	75793
❑	2-Propanol, anhydrous, 99.5% (Isopropanol)	Sigma-Aldrich	278475

Table 1.11 Reagents and Gels Required to Run QC Steps

✓	Item	Supplier	Part Number
❑	Mother E-Base™ Device	Thermo Fisher Scientific (formerly Life Technologies™ /Invitrogen)	EB-M03
❑	Daughter E-Base™ Device		EB-D03
❑	E-Gel® 48 4% agarose gels (for Sample QC)		G8008-04
❑	TrackIt™ 25 bp DNA Ladder (for Sample QC)		10488-022
❑	TrackIt™ Cyan/Orange Loading Buffer (for Sample 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
❑	E-Gel® 48 2% agarose gels (optional mPCR QC)		G8008-02
❑	Water, nuclease-free, ultrapure MB grade	Affymetrix	71786
❑	50 bp DNA ladder (optional mPCR QC)	New England BioLabs Inc.	N3236S

Other Lab Equipment Required

The remaining equipment required is listed in [Table 1.12](#).

Table 1.12 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 and PCR strip tube adapter*	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
❑	Zerostat Anti-static Gun	Affymetrix	74-0014

* Equivalent items from other manufacturers are acceptable.

Appendix A

Supplier Contact List

Table A.1 Supplier Contact List

Supplier	Website Address
Affymetrix	www.affymetrix.com
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
New England BioLabs Inc.	www.neb.com
QIAGEN	www.qiagen.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)	www.thermofisher.com
USB Corporation (Affymetrix)	www.usb.affymetrix.com
VWR	https://us.vwr.com/store/
Biometra	www.biometra.de