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CarrierScan[™] Assay 96-Array Format Automated Workflow SITE PREPARATION GUIDE

for Biomek FXP (Windows® 7)

Catalog Number 951951
Publication Number 703480
Revision 3





Affymetrix Pte Ltd 7 Gul Circle #2M-01 Keppel Logistics Building Singapore 629563 Products:

CarrierScan™ 1S 96F Array Plate



Thermo Fisher Scientific Baltics UAB V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania **Products:**

CarrierScan™ Reagent Kit

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. 703480

Revision	Date	Description
3	16 October 2020	Updated document to reflect new names for reagent kit components. Added the plate option of Bio-Rad HSP9601 as an alternative for the Bio-Rad HSP-9631 plate.
2	22 May 2018	Updating to add new CarrierScan™ 1S 96F Array Plate. Includes an option for a three-hour DNA precipitation step to enable faster assay turnaround time.
1	24 October 2017	New publication.

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Beckman Coulter Biomek® FXP Target Prep Express configuration

Introduction

CarrierScan[™] Assay 96-Array Format Automated Workflow described in the *CarrierScan*[™] Assay 96-Array Format Automated Workflow for Biomek FX^P User Guide (Pub No. 703478) leverages the Axiom 2.0 Assay method on the Biomek FX^P for target preparation and GeneTitan reagent preparation. The multiplex PCR (mPCR) and mPCR spike-in steps are not part of the automated method and must be executed off-deck, manually.

Equipment

The Biomek® FXP Target Prep Express (Cat. No. A83103) is required to run the CarrierScan Assay with the automated target preparation. This workstation includes the accessories, software and deck configuration listed in Table 1. For information on upgrading a Biomek liquid handler already at your site, contact Beckman Coulter. In addition to this workstation, the components listed in Chapter 2 are also required. This chapter details the configuration required when using Windows® 7 operating system.



Table 1 Biomek FXP Target Prep Express required for running the CarrierScan Assay Automated Workflow

✓	Item	Quantity	Beckman Coulter Cat No.
	Biomek FX ^P Target Prep Express The catalog number for this workstation includes the components listed below.	1	A83103
	Biomek FX ^P Dual Multichannel Span-8	1	A31844
	Biomek Windows 7 New Installation kit with Biomek Software 4.1	1	B40917
	Packaged Tip Loader	1	719356
	Span-8 Disposal ALP	1	719590
	Low Volume Disposable Mandrel Kit 8-Pack	1	A39380
	BFX 96-channel Disp, Tip Head, 200U	1	719368
	Pkg Static ALP Platform	4	719357
	• 4 × 3 ALP Kit	1	719948
	Static Peltier ALP	1	A93938
	Shaking Peltier ALP	1	A93942
	Adapter, Deepwell Plate (required for the assay)	1	A83050
	Adapter, Thin PCR (required for HT 3' IVT PLUS and HT WT PLUS assays)	1	A19709
	Frame for Modular Reservoir	4	372795
	• 24-Position Tube Rack	1	373661
	• Inserts, Tube, 11 mm (case of 25)	1	373696
	24 Position Cold Tube Block (referred to as Reagent Cold Block in this document)	1	A83054
	Biometra T-Robot¹ Integration - v4.1	1	969125

¹ The thermal cycler for the Biomek® FXP Target Prep Express must be purchased separately. Beckman Coulter will provide the services for integrating the thermal cycler on to the deck of the Biomek FXP Target Prep Express. The warranty and service for the thermal cycler must be purchased separately from the manufacturer/distributor of the thermal cycler.

Instrument specifications and settings

In order for the CarrierScan Assay to be successfully performed on the Biomek FX^P Target Prep Express Instrument the system must meet the following specifications and be properly configured.

- Biomek FX^P Target Prep Express system
 - Hybrid dual armed Biomek FXP
 - 96MC head left pod and Span-8 head right pod
 - 1 mL syringes and low volume tubing on the Span-8 system
 - Deepwell plate adapter mounted on the Shaking Peltier Device
 - ALPs secured to deck as shown in Figure 1
- System fluid is degassed water
- Clean compressed air is at 40–80 psi
- Computer workstation specifications
 - Windows® 7 operating system
 - Internet Explorer® v8.0
 - Microsoft® Excel® 2010 software; 32-bit version ONLY software
 - Biomek software version 4.1 (Build: 41.0)
- · Biomek software
 - Make sure that the Affymetrix Deck is the default deck
 - Make sure deck location SPelt96_1 has the device ShakingPeltier1 associated with it
 - Make sure deck location Pelt_1 has the device StaticPeltier1 associated with it
 - Make sure deck location TR1 is ALP type Span8TrashRight and not Span8TipTrashRight
- Check for a properly framed deck for both the Biomek FX^P 96MC and Span-8 pods
 - Framing tolerance is 0.03 cm. Reframe if this is exceeded when checked
 - If deck was reframed, save a current copy of the instrument file for backup
 - Exit and restart Biomek software after installing Affymetrix Deck and after any changes in deck framing in order to save the new values in the default instrument (.bif) file
- Additional Pod Settings
 - Navigate to: Instrument → Hardware Setup → Pod2 → Additional Pod Settings
 - Pod 2 (Span-8) settings: Set System Trailing Airgap to 20 μL (Figure 2)
 - Pod 1 (MC96) settings (Figure 3):
 - Check **Always Rove at Z-Max** box

Set Additional Roving Height to 0.45 cm or lower (default is 0.75 cm) Set Gripper Offset in D Axis to 0.7 cm



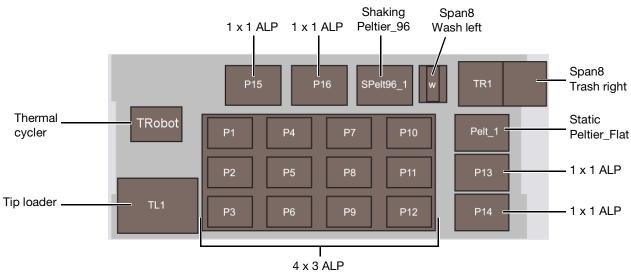


Figure 1 Biomek FX^P TPE system (top) and deck configuration (bottom)



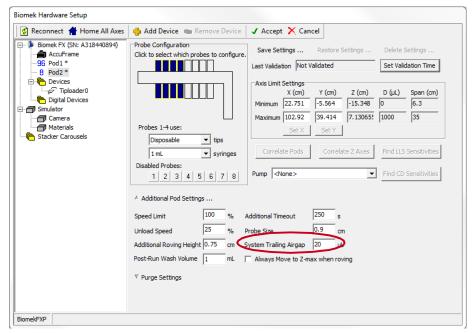


Figure 2 Hardware setup changes for Span-8 Pod2

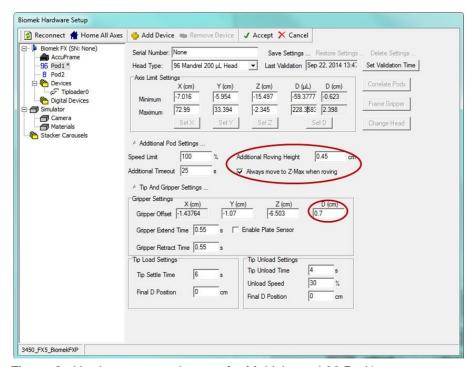


Figure 3 Hardware setup changes for Multichannel 96 Pod1

Importing the Axiom™ 2.0 Target Prep Method

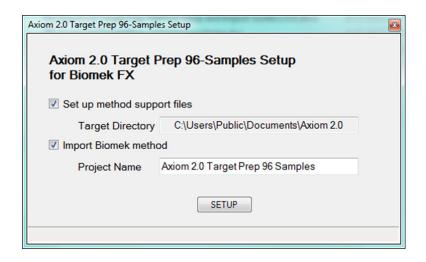
The purpose of this section is to identify the critical steps for importing the Axiom 2.0 Target Prep method for Windows® 7 into the Biomek® software for use on the Biomek® FXP Target Prep Express.

Importing the method

- 1. Run Setup.exe.
- 2. Click **Setup.exe** in method package to copy files from the package to the following locations and import the Biomek method.

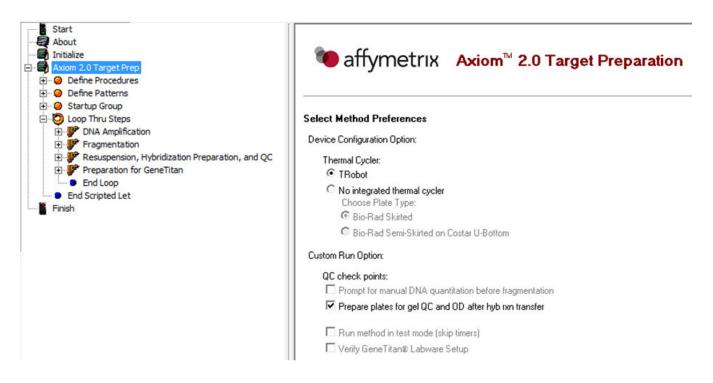
Table 2 Directory location

Source	Destination	
\Root*.*	C:\Users\Public\Documents\Affymetrix\Axiom 2.0	
\Images\	C:\Users\Public\Documents\Axiom 2.0\Images	
\Misc\	C:\Users\Public\Documents\Axiom 2.0	



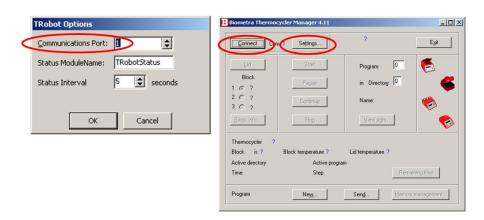
3. Set Method Preferences:

a. Locate and click the method branch named **Axiom 2.0 Target Prep**.



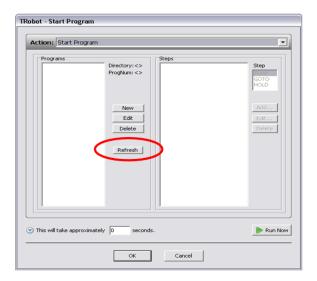
- b. Select the thermal cycler setting that matches the configuration used on the system.
- c. Set the "Test Mode" run option to disable.
- 4. Load Thermocycler Programs onto TRobot.
 - a. Click Start → Programs → Thermocycler Manager 4.11 → Thermocycler Manager 4.11 to open the TRobot Thermocycler Manager software.
 - b. Set COM port for TRobot device and connect. Find TRobot COM port setting at the following location in Biomek Software:

Device Editor → **Configuration Options** → **TRobot**.





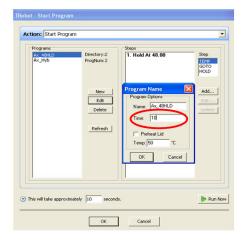
- 5. Load programs to device.
 - a. Click Memory Management in the lower right hand corner of the **Biometra Thermocycler Manager 4.11** window.
 - b. Click **Download Programs from PC** to load programs into the device. Select all of the *.tpb files from the source location (2 files total).
 - C:\Users\Public\Documents\Affymetrix\Axiom 2.0\TRobot Protocols
- 6. Refresh the list in TRobot SILAS Module.
 - a. Go to **Device Editor** → **TRobot**. Click the **Refresh** button. The list takes at least a minute to populate.



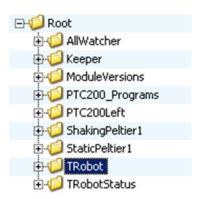
7. Enter the estimated time for each temperature program.

Table 3 Estimated program times

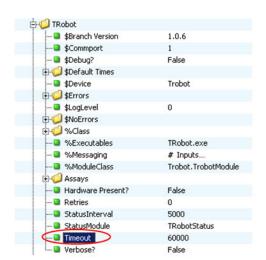
File Name	Program name	Time
Ax2_Hyb_2_3.tpb	Ax2_Hyb	953
Ax_48HLD_2_2.tpb	Ax_48HLD	10



- 8. Change TRobot Module timeout setting in SILAS Keeper Editor to 180000 ms:
 - a. Click Start → Programs → Beckman Coulter → Service → SILAS Keeper Editor.
 - b. Locate the **TRobot** module branch and expand.

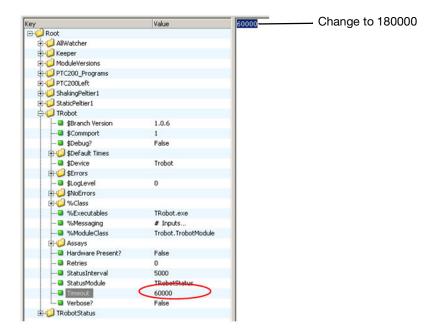


c. Locate and select the **Timeout** setting.





d. Change the **Timeout** setting to "180000" ms in panel on the right.





Equipment and supplies required

This site preparation guide includes the supplier and catalog number information for the equipment, software, reagents, arrays, labware, and other consumables that have been verified for use with the CarrierScan[™] Assay Automated Workflow.

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

Table 4 Thermo Fisher Scientific equipment, software, reagents and arrays required

✓	Item	Source
Equip	oment	
	GeneTitan [™] Multi-Channel Instrument ¹	Contact Thermo Fisher Scientific, Inc.
Softv	vare	
	GeneChip™ Command Console™ (GCC)	version 4.3 or later
	Reproductive Health Research Analysis Software (RHAS)	version 1.0 or later
Reag	ents	
	CarrierScan™ Reagent Kit 96 Reactions CarrierScan Reagent Kit 96 Reactions contains the following modules for processing one 96- array format plate: • CarrierScan mPCR Module (qty one) • Module 1 (quantity one) • Module 2-1 (quantity one) • Module 2-2 (quantity one) • Module 3-1 (quantity one) • Module 3-2 (quantity one) • Wash Buffer A, two bottles • Wash Buffer B, one bottle • Water, one bottle	931933 • 931939 • 906011 • 906012 • 906013 • 906014 • 906022 • 906023 • 906020
Array		
	CarrierScan™ 1S 96F Array Plate	951950

Table 4 Thermo Fisher Scientific equipment, software, reagents and arrays required (Continued)

✓	Item	Source
Carri	erScan kits	
	CarrierScan [™] 1S Assay Kit Each Kit Includes: • CarrierScan [™] Reagent Kit 96 Reactions • One CarrierScan [™] 1S 96F Array Plate • One Axiom [™] GeneTitan [™] Consumables Kit	951951
Carri	CarrierScan™ 1S Training Kit Each Kit Includes: • CarrierScan™ Reagent Kit 96 Reactions (enough to process 2 x 96-format array plates) • Reference gDNA 103 (2 vials) • Two CarrierScan™ 1S 96F Array Plates • Two Axiom™ GeneTitan™ Consumables Kits • Two CarrierScan™ DNA Training Plates, for 96-format assay erScan™ Array GeneTitan™ consumables Each Axiom™ GeneTitan™ Consumables Each Axiom™ GeneTitan™ Consumables Kit contains the following labware for each array plate: • One hybridization tray • One scan tray with cover and protective base • Five stain trays with covers These trays are required for processing array plates on the GeneTitan™ Multi-Channel Instrument.	951955 901606
Othe		
	Axiom 96 Consumable Kit for Biomek FXP (Sufficient for 4 x 96 rxn runs)	902800
	Axiom 96 Consumable Kit for QC (Sufficient for 40 x 96 rxn runs)	902801
	Axiom 96 Consumable Kit for off-deck (Applied Biosystems) TC (Sufficient for 5 x 96 rxn runs)	902803
	Axiom 96 TC Plate Sealing Kit (Sufficient for 25 x 96 rxn runs)	902802
	Axiom 2.0 Target Prep Express Templates Kit for Windows® 7 Note: Available for the CarrierScan Assay automated target prep only, and is to be ordered by the Thermo Fisher Scientific FAS prior to beginning training.	904006

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* (Pub. No. 08-0305).

Labware and reagents required for mPCR preparation and mPCR quality control gel protocol

 Table 5
 Labware and reagents for mPCR preparation and mPCR gel QC

✓	Description	Item	Supplier	Cat No.
	mPCR Sample Plate	For Applied Biosystems thermal cyclers: Bio- Rad Hard-Shell Full-Height 96-Well Semi- Skirted PCR Plate	Bio-Rad	• HSS-9641
		For Eppendorf Mastercycler pro S: Bio-Rad Hard-Shell Low Profile 96-well Full-Skirt PCR Plate		• HSP-9631 or HSP-9601
	mPCR Master Mix reservoir	Matrix™ 25-mL reservoir	Thermo Fisher Scientific	8093-11
	mPCR Master Mix tube	15-mL conical centrifuge tube, polypropylene, sterile	Various	Various
	mPCR kit	QIAGEN Multiplex PCR Plus Kit (100)	QIAGEN	206152
	mPCR QC gel	E-Gel® 48 2% agarose gels	Thermo Fisher Scientific	G8008-02
	mPCR QC ladder	50 bp DNA ladder	New England BioLabs Inc.	N3236S
	mPCR QC dilution buffer	Reduced EDTA TE Buffer (10 mM Tris-HCL PH 8.0, 0.1 mM EDTA)	Thermo Fisher Scientific	75793
	mPCR Dilution QC plate mPCR Gel QC plate	96-well PCR plate (for mPCR QC)	Various	Various

Labware required for the Biomek FXP Target Prep Express

The labware required to run the CarrierScan Assay Automated Workflow on the Biomek FX^P Target Prep Express is listed in Table 6. Photographs of the labware are provided in Table 13 on page 20.

Note: The tips and universal labware for the Biomek FX^P available from Beckman are also available as a single sales kit. The sales kit (Cat. No. A87508) contains labware sufficient for processing ten array plates. The labware can be ordered as individual cases from the Beckman eStore.

Table 6 Labware for the Biomek FXP Target Prep Express

✓	Item	Quantity required 96-array plate run ¹	Supplier	Cat No.			
Pipe	tte Tips, barrier, 96 tips/rack						
	Biomek Span P50, pre-sterile, barrier	96 tips		A21586			
	Biomek AP96, P250, pre-sterile, barrier	384 tips	Beckman Coulter	717253			
	Biomek Span P250, pre-sterile, barrier	51 tips		379503			
	Biomek Span P1000, pre-sterile, barrier, conductive	188 tips		987925			
Plate	Plates						
	Bio-Rad Hard-Shell 96-well	9	Bio-Rad	HSP-9631 or HSP-9601			
	Beckman Deepwell Titer, polypropylene	2	Beckman Coulter	267007			
	Eppendorf 96 Deepwell Plate, 2,000 µL	1	Eppendorf	951033481			
	Plate, OD for UV spec, 96-well	1	E & K Scientific	EK-25801			
Reservoirs, modular for reagents							
	Half module, 75-mL capacity	3	5	372786			
	Quarter module, 40-mL capacity	12	Beckman Coulter	372790			
	Quarter module, divided by width, 19-mL capacity	5		372792			

¹ For pipette tips, the "Quantity required" column lists the total number of tips required and not the number of racks.

Labware consumables kit for target preparation

The following consumable kits for on-deck (TRobot) and off-deck (Applied Biosystems, PTC, or Eppendorf) thermal cycler users are now available to order through Thermo Fisher Scientific. Please refer to Table 11 "Guidance for consumable kit ordering" to determine which kits are specific to your needs.

- Axiom 96 Consumable Kit for Biomek FX^P (Cat. No. 902800)
- Axiom 96 Consumable Kit for QC (Cat. No. 902801)
- Axiom 96 Consumable Kit for Off-Deck Applied Biosystems TC (Cat. No. 902803)
- Axiom 96 TC Plate Sealing Kit (Cat. No. 902802)

Note: Beckman plates, reservoirs, and tips are not included in the kits prepared by Thermo Fisher Scientific. You must order all Beckman supplies directly from Beckman.

Table 7 Axiom 96 Consumable Kit for Biomek FX^P (Cat. No. 902800)

Labware item	Part No.	Quantity
Bio-Rad Hard-Shell 96-well Plate	203015	40
Eppendorf 96 Deepwell Plate, 2,000 μL	203079	4

Table 8 Axiom 96 Consumable Kit for QC (Cat. No. 902801)

Labware item	Part No.	Quantity
OD Plate, UV	202609	40

Table 9 Axiom 96 Consumable Kit for off-deck Applied Biosystems TC (Cat. No. 902803)

Labware item	Part No.	Quantity
Bio-Rad Hard-Shell 96-well semi-skirted PCR Plate	203055	5
Costar Clear Polystyrene 96-Well Plates	202165	5

Table 10 Axiom 96 TC Plate Sealing Kit (Cat. No. 902802)

Labware item	Part No.	Quantity
Bio-Rad Metal Lid	202519	1
Bio-Rad 'P' Pad	202958	1

Table 11 Guidance for consumable kit ordering

	Thermal cycler option			
Consumable kit		Off-deck PTC-200	Off-deck Applied Biosystems	Off-deck Eppendorf Mastercycler pro S
Axiom [™] 96 Consumable Kit for Biomek FX ^P (Cat. No. 902800)	✓	✓	✓	✓
Axiom [™] 96 Consumable Kit for QC (Cat. No. 902801)	✓	✓	✓	✓
Axiom [™] 96 Consumable Kit for Off-Deck Applied Biosystems TC (Cat. No. 902803)			✓	
Axiom™ 96 TC Plate Sealing Kit (Cat. No. 902802)	✓			

Table 12 Other consumables required

✓	Item	Manufacturer/ Distributer	Cat. No.		
	Microcentrifuge tubes and tube holder	various	various		
	50-mL conical centrifuge tube, polypropylene, sterile	various	various		
	Tube holder, 15 and 50 mL	various	various		
	96-well PCR plate rack	various	various		
	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	Thermo Fisher Scientific Bio-Rad	4306311 MSB1001		
	Kimwipes®	various	various		
	Adhesive film for 96-well plates—use one of the following:				
	MicroAmp® Clear Adhesive Film	Thermo Fisher Scientific	4306311		
	Microseal 'B' Film	Bio-Rad	MSB1001		
	Markers, permanent, fine point	various	various		

Images of the labware and materials required

The photographs in Table 13 are provided to help visualize the labware required on the Biomek FX^P Target Prep Express to run the automated target preparation portion of the CarrierScan Assay Automated Workflow.**deck**

Table 13 Labware and materials used on the Biomek workstation deck

Labware	Supplier and Cat. No.	Image
Biomek AP96 – P250 Pipette Tips (aqua box; pre-sterile, barrier)	Beckman Coulter Cat. No. 717253	
Biomek Span P250 Pipette Tips (green box; pre-sterile, barrier)	Beckman Coulter Cat. No. 379503	
Biomek Span P1000 Pipette Tips (yellow box; pre-sterile, barrier, conductive)	Beckman Coulter Cat. No. 987925	
Biomek Span P50 Pipette Tips (pink box; pre-sterile, barrier)	Beckman Coulter Cat. No. A21586	

Table 13 Labware and materials used on the Biomek workstation deck (Continued)

Labware	Supplier and Cat. No.	Image
Bio-Rad Hard-Shell 96-well Plate (available in multiple colors)	Part of the Axiom [™] 96 Consumable Kit for Biomek FXP Cat. No. 902800 Plate Part No. 203015 Alternate Vendor: Bio-Rad, Cat. No. HSP-9631 (blue) or HSP-9601 (white)	A 2 3 4 5 6 7 8 9 10 11 12 B 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Eppendorf 96 Deepwell Plate, 2,000 μL, (available in multiple colors)	Part of the Axiom [™] 96 Consumable Kit for Biomek FX ^P Cat. No. 902800 Plate Part No. 203079 Alternate Vendors: Eppendorf, Cat. No. 951033481	A 1 2 3 4 5 6 7 8 9 10 11 12 A B B C C D B B B B B B B B B B B B B B B
OD Plate, UV	Part of the Axiom™ 96 Consumable Kit for QC Cat. No. 902801 Plate Part No. 202609 Alternate Vendor: Thomas Scientific EK-25801	A O O O O O O O O O O O O O O O O O O O

 Table 13
 Labware and materials used on the Biomek workstation deck (Continued)

Labware	Supplier and Cat. No.	Image
Lid, metal (arched, auto-sealing with P pads)	Part of the Axiom™ 96 TC Plate Sealing Kit Cat. No. 902802 Lid Part No. 202519 P Pad Part No. 202958 Alternate Vendor: Bio-Rad Cat. No. MSL-2032 and P Pad Cat. No. MSP-1003	Front view Side view
Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate This consumable is required only if using off-deck Applied Biosystems 2720 or Applied Biosystems 9700 Thermal cyclers	Part of the Axiom [™] 96 Consumable Kit for Off- deck (Applied Biosystems) TC Cat. No. 902803 Plate Part No. 203055 Alternate Vendor: Bio-Rad Cat. No. HSS-9601	
Plate, Costar Brand Serocluster round Bottom Plate from Corning Note: this consumable is required only if using an off-deck Applied Biosystems 9700 or Applied Biosystems 2720 thermal cycle	Part of the Axiom™ 96 Consumable Kit for Off- deck (Applied Biosystems) TC Cat. No. 902803 Plate Part No. 202165 Alternate Vendors: VWR International Cat. No. 29442-392 E&K Scientific Cat. No. EK 680568 Corning Mfg Cat. No. 3795	A

Table 13 Labware and materials used on the Biomek workstation deck (Continued)

Labware	Supplier and Cat. No.	Image
The Applied Biosystems 9700 and to use the semi-skirted 96-well plates on the Biomek deck, the semi-skirt stacked on a Costar brand Seroclu Microtitration plate as shown in the	(Cat. No. HSS-9601). For use ed PCR plate must be ster 96-well Round Bottom	
Beckman deepwell titer plate (polypropylene)	Beckman Coulter Cat. No. 267007	
Frame for reservoirs	Beckman Coulter Cat. No. 372795	
Half reservoir Half module, 75 mL capacity	Beckman Coulter Cat. No. 372786	



 Table 13
 Labware and materials used on the Biomek workstation deck (Continued)

Labware	Supplier and Cat. No.	Image
Quarter reservoirs • Quarter module, 40 mL capacity • Quarter module divided by width, 19 mL capacity each receptacle	Beckman Coulter • Cat. No. 372790 (40 mL) • Cat. No. 372792 (19 mL)	Undivided 40 mL capacity Divided by width 19 mL capacity
Reagent block, chilled to 4°C	Beckman Coulter Cat. No. A83054	Metal posts on block circled in red.
24-position tube rack with one 11 mm tube insert in position A6.	Beckman Coulter Cat. No. 373661 (rack) Cat. No. 373696 (insert)	Tube insert A6

Table 13 Labware and materials used on the Biomek workstation deck (Continued)

Labware	Supplier and Cat. No.	Image
Adaptor, deepwell plate (installed on the Shaking Peltier)	Beckman Coulter Cat. No. A83050	The metal block is the adaptor.
This adaptor is typically installed by a Beckman Coulter field service technician during new system installation or a system upgrade. Ensure that you have 1 of these adaptors on the deck prior to running this assay.		
Reagent block template (designed specifically for use with the CarrierScan Reagent Kit)	Contact Thermo Fisher Scientific	Template on reagent block. Metal posts on block circled in red. Axiom™ 2.0 Reagent Kill Axiom™ 2.0 Reagent Kill
Zerostat Anti-static Gun and Ion-Indicator Cap	Milty Zerostat, Thermo Fisher Scientific Cat. No. 74-0014	ZEROSTAT 3 MILTY Caddors Map on of meach of shibban

The reagent block template (Part No. 15-0439) and reservoir frames template stickers (Part No. 101135) are provided in the Axiom 2.0 Target Prep Express Templates Kit for Windows® 7 (Cat. No. 904006) and are ordered by your Thermo Fisher Scientific FAS prior to beginning training. These templates are only used with the automated target preparation protocol.

Reagent block template

The Axiom reagent block template was designed to fit precisely onto the top of the Beckman Coulter Tube Block (Cat. No. A83054). It is held in place by the metal posts on the block (Figure 4). Using this template will help ensure the proper placement of reagent tubes onto the block for each method.

Reagent block

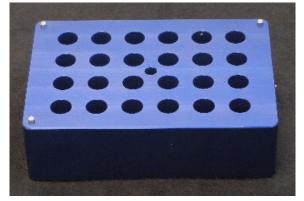
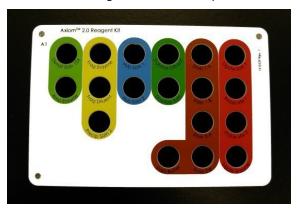


Figure 4 Axiom reagent template for tube block

Reagent block with template



Reservoir labels

The reservoir labels are stick-on labels for the modular reagent reservoir frames (used at different stages in the automated workflow) and are provided in the Axiom™ 2.0 Target Prep Express Templates Kit for Windows® 7. These stick-on labels are color-coded to match the colors found on the caps of the reagent tubes in the CarrierScan Reagent Kit. Using these labels helps ensure the proper placement of reservoirs and reagents for each method.

There are four reservoir holders used in the Axiom 2.0 method. Three of these will have templates on two sides and the remaining reservoir holder will have a template on one side for a total of seven templates.

Remove the protective surface from the back of the label and place on the reservoir frames as directed in the Figure 5 through Figure 8.

Reservoir frame 1

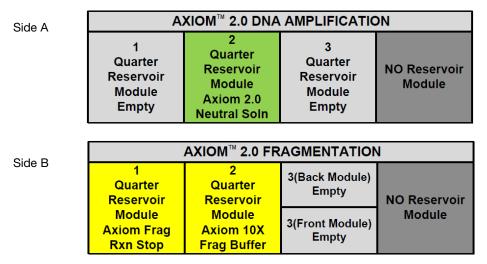


Figure 5 Reservoir frame 1 for Windows® 7 users

Reservoir frame 2

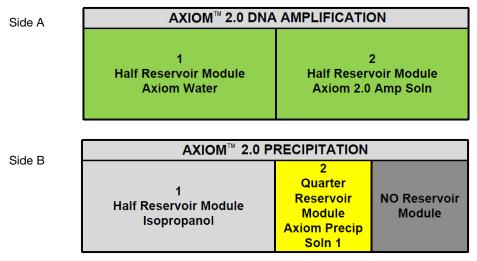


Figure 6 Reservoir frame 2 for Windows® 7 users

Reservoir frame 3

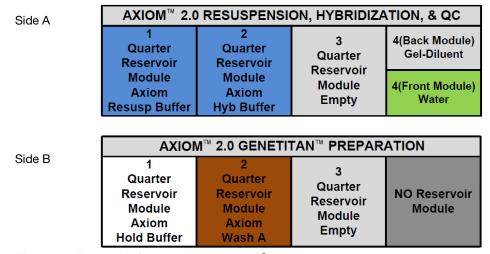


Figure 7 Reservoir frame 3 for Windows® 7 users

Reservoir frame 4

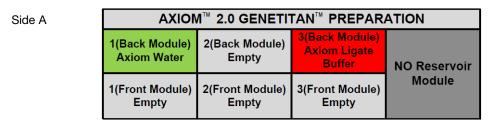


Figure 8 Reservoir frame 4 for Windows® 7 users

Other equipment, consumables and reagents required

Pre-amplification area

Precautions are required when manipulating genomic DNA to avoid contamination with foreign DNA amplified in other reactions and procedures. It is recommended that genomic DNA manipulations are performed in a dedicated pre-amplification room or area separate from the main laboratory.

This pre-amplification area should have a dedicated set 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.

Oven requirements for automated target preparation

We recommend using the ED 56 BINDER oven listed in Table 14. If another oven is used, it must be able to maintain a constant temperature of 37° C for at least 24 hr, and have a temperature accuracy of $\pm 1^{\circ}$ C.

 Table 14
 Ovens required for the CarrierScan Assay Automated Target Preparation

✓	Item	Supplier	Cat. No.
	Oven: Required if processing more than 3 array plates per week. • ED 56 Drying and Heating Chamber¹	BINDER	ED056UL-120V Voltage: 120 V 1~60 Hz Cat. No. 9010-0334 ED056-230V Voltage: 230 V 1~50/60 Hz Cat. No. 9010-0333
	Optional — for low throughput of 3 or fewer array plates per week: • Applied Biosystems™ GeneChip™ Hybridization Oven 645²	Thermo Fisher Scientific	00-0331

¹ Replaces BINDER Model ED 53.

Spectrophotometer

We recommend using one of the spectrophotometers listed in Table 15.

 Table 15
 Spectrophotometers

✓	Item	Supplier	Cat. No.
	One of the following spectrophotometers: • DTX 880 Multimode Detector, with: Genomic Filter Slide	Beckman Coulter	987921 – detector A30184 – filter slide
	SpectraMax® High Throughput Microplate Spectrophotometer	Molecular Devices	Plus384

² The GeneChip[™] Hybridization Oven 640 is not supported with the CarrierScan Assay.

Thermal cycler recommendations and protocols

We have verified the performance of this assay using the thermal cyclers listed below in their 96-well block configurations:

Table 16 Thermal cycler guidance

	Thermal cycler program		
Verified thermal cyclers	CarrierScan mPCR	CarrierScan Denature	
Biometra TRobot 96 (on-deck)	No	✓	
Applied Biosystems 2720	No	✓	
BioRad/MJ DNA Engine Tetrad® 2 PTC-0240G	No	✓	
Applied Biosystems 9700 (with a gold or silver block)	✓	√	
Applied Biosystems Veriti	✓	✓	
Applied Biosystems ProFlex	✓	✓	
Eppendorf® Mastercycler® pro S	✓	✓	

IMPORTANT! Always use the heated lid option when programming protocols. The **CarrierScan mPCR** protocol was verified using the "9600 mode" on the Applied Biosystems 9700, Applied Biosystems Veriti, and Applied Biosystems ProFlex thermal cyclers. The "Safe" mode was used for the Eppendorf Mastercycler pro S. Refer to the manufacturer's user guide for programming information.

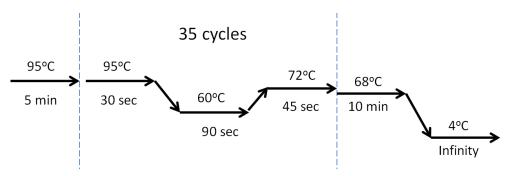


Figure 9 CarrierScan mPCR thermal cycler protocol (Stage 1A)

The mPCR step of the CarrierScan Assay has been verified with the Applied Biosystems 9700 (with gold-plated or silver block) Applied Biosystems Veriti, Applied Biosystems 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.

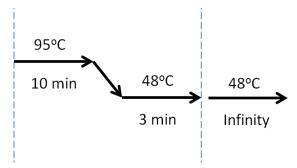


Figure 10 CarrierScan Denature thermal cycler protocol (Stage 4)



WARNING! Evaporation during denaturation can negatively impact assay performance. Use the recommended thermal cycler consumables and sealing film to eliminate condensation and evaporation. For thermal cyclers with variable lid tension (such as the Bio-Rad PTC-200 or Tetrad 0240) please follow the manufacturer's instructions for adjusting lid tension.

Note: Two thermal cylers are required if running the three plate/week or eight plate/week automated target preparation workflow.

PCR plate type by thermal cycler for mPCR

Table 17 provides details into the consumables to be used with each thermal cycler when executing the mPCR step.

Table 17 PCR plate type by thermal cycler for mPCR

Thermal cycler model	PCR plate type	Seal ¹
Applied Biosystems 9700	Bio-Rad Hard-Shell Full-Height 96-Well Semi- Skirted PCR Plate (Cat. No. HSS-9641)	MicroAmp [™] Clear Adhesive Film from Applied Biosystems (Cat. No. 4306311)
		MicroAmp™ Clear Adhesive Film from Applied Biosystems (Cat. No. 4306311)
Applied Biosystems ProFlex	Bio-Rad Hard-Shell Full-HCat. No. HSS-9641)	MicroAmp™ Clear Adhesive Film from Applied Biosystems (Cat. No. 4306311)
Eppendorf Mastercycler pro S	Bio-Rad Hard-Shell Low Profile 96-well Full- Skirt PCR Plate (Cat. No. HSP-9631 or HSP- 9601)	MicroAmp™ Clear Adhesive Film from Applied Biosystems (Cat. No. 4306311)

¹ Microseal 'B' film from Bio-Rad (Cat. No. MSB-1001) may be used in place of MicroAmp Clear Adhesive Film for the Applied Biosystems thermal cycler.

Shakers

We recommend using one of the following shakers listed in Table 18.

Table 18 Shakers

Shaker	Supplier
Thermo Scientific™ Digital Microplate Shaker	120V US/JP plug (Cat. No. 88882005) or Digital Microplate Shaker, 230V EU/UK/ CHN Plug (Cat. No. 88882006).
Thermo Scientific™ Compact Digital Microplate Shaker	88880023
Jitterbug™	Boekel Scientific, Model 130 000

Plate centrifuge

One plate centrifuge is required for the Carrierscan Assay Automated Workflow. We recommend the plate centrifuges listed in Table 19. When centrifuging and drying pellets, the centrifuge must be able to spin down plates at:

- rcf: 3,200 x g with an appropriate rotor bucket combination (4,000 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 deepwell 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 19
 Plate centrifuges recommended for the CarrierScan Assay Automated Workflow

✓	Item	Supplier	Cat No.
	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:

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

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

Other common lab equipment required

Table 20 Other equipment required

✓	Item	Supplier	Cat No.
	Freezer, -20°C	MLS	_
	Refrigerator, 2–8°C	MLS	_
	Vortex-Genie® (for plates and microtubes)¹	Scientific Industries	SI-0236 (120 V/60 Hz) SI-0246 (230 V/50 Hz)
	Mini Microcentrifuge, for 2-mL tubes ¹	VWR	93000-196 (120V) 93000-196 (230V)
	Bel-Art Cryo-Safe Mini Cooler, -15°C1	VWR	47751-730
	Ice bucket, 4 to 9 liters	MLS	_
	Pipet-Aid ¹	VWR	53106-220
	Zerostat Anti-static Gun	Thermo Fisher Scientific	74-0014

 $^{^{\}mbox{\scriptsize 1}}$ Equivalent items from other manufacturers are acceptable.

Other reagents and gels required

Table 21 Other reagents required

✓	Item	Supplier	Cat. No.
	Quant-iT PicoGreen dsDNA Assay Kit (recommended for DNA quantitation)	Thermo Fisher Scientific	P7589
	2-Propanol, anhydrous, 99.5% (Isopropanol)	Sigma-Aldrich	278475

Table 22 Reagents and gels required to run QC steps

✓	Item	Supplier	Cat. No.
	Mother E-Base™ Device		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)	Thermo Fisher Scientific	10488-022
	TrackIt™ Cyan/Orange Loading Buffer (for Sample QC) or similar product		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 (for Sample QC)		71786

Pipettes and tips

Pipettes and tips recommended for performing the gel QC steps in the CarrierScanTM Assay Automated Workflow are listed in Table 23.

Table 23 Recommended pipettes and tips for CarrierScan Assay 96-Array Format Protocol

✓	Equipment	Manufacturer /Distributor	Cat. No.
	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

Documentation and support

Related documentation

Table 24 Documents related to the CarrierScan[™] Assay 96-Array Format Automated Workflow for Biomek FX^P

Document	Publication number	Description	
CarrierScan [™] Assay 96-Array Format Automated Workflow for Beckman Biomek FX ^P (Windows [®] 7) Site Preparation Guide	703480	Provides guidance on reagents, instruments, and supplies required to run the CarrierScan Assay 96-Array Format Automated Workflow for Biomek FXP.	
CarrierScan [™] Assay 96-Array Format Automated Workflow for Beckman Biomek FX ^P (Windows® 7) Quick Reference	703479	An abbreviated reference for the target preparation step of the CarrierScan Assay 96-Array Format Automated Workflow for Biomek FXP running on the Windows 7 operating system. This quick reference document is intended for experienced users.	
Axiom [™] 2.0 gDNA Sample Preparation Protocol QR	702987	An abbreviated reference on the genomic DNA sample preparation protocol.	
GeneTitan [™] MC Protocol for Axiom [™] 2.0 Array Plate Processing QR	702988	An abbreviated reference for processing Axiom 2.0 array plates with the GeneTitan Multi-Channel Instrument.	
GeneTitan™ Multi-Channel Instrument User Guide	08-0308	The GeneTitan Multi-Channel (MC) Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the art imaging device into a single bench-top instrument. This document detailing the use, care, and maintenance for the GeneTitan MC Instrument.	
GeneTitan™ Multi-Channel Instrument Site Preparation Guide	08-0305	Provides guidance on creating and maintaining the proper environment required for the GeneTitan Multi-Channel Instrument.	

Table 24 Documents related to the CarrierScan[™] Assay 96-Array Format Automated Workflow for Biomek FX^P

Document	Publication number	Description
Analysis and Software		
Axiom™ Genotyping Solution Data Analysis Guide	702961	This guide provides information and instructions for analyzing Axiom genotyping array data. It includes the use of Axiom™ Analysis Suite, Applied Biosystems Microarray Power Tools (formerly APT) and SNPolisher R package to perform quality control analysis (QC) for samples and plates, SNP filtering prior to downstream analysis, and advanced genotyping methods.
Applied Biosystems [™] GeneChip [™] Command Console [™] Software User Guide	702569	This user guide provides instructions on using Applied Biosystems GeneChip Command Console Software (GCC) used to control GeneChip instrument systems. Command Console Software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip Arrays.
Reproductive Health Research Analysis Software	703517	This user guide provides instructions on using Applied Biosystems™ Reproductive Health Research Analysis Software (RHAS), a single source comprehensive software package to enable the QC, copy number, genotyping, variant status determination and SMN screening of microarray data designed for Reproductive Health research applications.
CarrierScan Assay Manual Protocol		
CarrierScan™ Assay 96-Array Format Manual Workflow User Guide	703481	This user guide provides comprehensive instructions on running the CarrierScan Assay 96-Array Format Manual Protocol
CarrierScan [™] Assay 96-Array Format Manual Workflow Site Preparation Guide	703483	Provides guidance on reagents, instruments, and supplies required to run the CarrierScan Assay 96-Array Format Manual Protocol.
CarrierScan™ Assay 96-Array Format Manual Workflow Quick Reference	703482	An abbreviated reference for the target preparation step of the CarrierScan Assay 96-Array Format Manual Protocol. This quick reference document is intended for experienced users.
Beckman Coulter documents		
Biomek [®] Liquid Handler User's Manual	987834	This document is installed at the same time as the Biomek FX ^P Target Prep Express software. To access, click Start → All Programs → Beckman Coulter → Manuals .
Biomek [®] Software User's Manual	B30026AA	This document is installed at the same time as the Biomek FX ^P Target Prep Express software. To access, click Start → All Programs → Beckman Coulter → Manuals .

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 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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