

CarrierScan™ Assay 96-Array Format Manual Workflow

SITE PREPARATION GUIDE

for use with:

CarrierScan™ 1S 96F Array Plate

CarrierScan™ Reagent Kit 96 Reactions

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Revision 3



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Products:
CarrierScan™ 1S 96F Array Plate



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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. 703483

Revision	Date	Description
3	15 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. Added the plate option of Abgene™ AB-0932 as an alternative for the Eppendorf 951033481 plate.
2	22 May 2018	Includes an option for a three-hour DNA precipitation step to enable faster assay turnaround time. Updating to add new CarrierScan™ 1S 96F Array.

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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 verified for use with the CarrierScan™ Assay 96-Array Format Manual Workflow.

For a list of related documentation, please refer to "[Documentation and support](#)" on page 15.

Equipment, software, reagents and arrays required

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

✓	Item	Source
Equipment		
<input type="checkbox"/>	GeneTitan™ Multi-Channel Instrument ¹	Contact Thermo Fisher Scientific, Inc.
Software		
<input type="checkbox"/>	GeneChip™ Command Console™ (GCC)	version 4.3 or later
<input type="checkbox"/>	Reproductive Health Research Analysis Software (RHAS)	version 1.0 or later
Reagents		
<input type="checkbox"/>	CarrierScan™ Reagent Kit 96 Reactions CarrierScan Reagent Kit 96 Reactions contains the following modules for processing one 96-array format plate: <ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • 931939 • 906011 • 906012 • 906013 • 906014 • 906015 • 906022 • 906023 • 906020

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

✓	Item	Source
Array		
<input type="checkbox"/>	CarrierScan™ 1S 96F Array Plate	951950
CarrierScan kits		
<input type="checkbox"/>	CarrierScan™ 1S Assay Kit Each Kit Includes: <ul style="list-style-type: none"> • CarrierScan™ Reagent Kit 96 Reactions • One CarrierScan™ 1S 96F Array Plate • One Axiom™ GeneTitan™ Consumables Kit 	951951
<input type="checkbox"/>	CarrierScan™ 1S Training Kit Each Kit Includes: <ul style="list-style-type: none"> • CarrierScan™ Reagent Kit 96 Reactions (enough to process 2 x 96-array format plates) • Axiom™ 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 	951955
CarrierScan™ Array GeneTitan™ consumables		
<input type="checkbox"/>	Each Axiom™ GeneTitan™ Consumables Kit contains the following labware for each array plate: <ul style="list-style-type: none"> • One hybridization tray • One scan tray with top cover and protective base • Five stain trays with top covers These trays are required for processing Axiom array plates on the GeneTitan™ Multi-Channel Instrument.	901606
Other kits		
<input type="checkbox"/>	Axiom 96 Consumable Kit for Biomek FX ^P (Sufficient for 4 x 96 rxn runs)	902800
<input type="checkbox"/>	Axiom 96 Consumable Kit for QC (Sufficient for 40 x 96 rxn runs)	902801
<input type="checkbox"/>	Axiom 96 Consumable Kit for off-deck (Applied Biosystems) TC (Sufficient for 5 x 96 rxn runs)	902803
<input type="checkbox"/>	Axiom 96 TC Plate Sealing Kit (Sufficient for 25 x 96 rxn runs)	902802
<input type="checkbox"/>	Axiom 2.0 Target Prep Express Templates Kit for Windows® 7 Note: Available for the Axiom 2.0 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, see the *GeneTitan Multi-Channel Instrument Site Preparation Guide* (Pub. No. 08-0305).

Equipment required for manual target preparation

Table 2 Additional instruments required for the CarrierScan™ Assay 96-Array Format Manual Workflow

✓	Equipment	Manufacturer/distributor
<input type="checkbox"/>	Two or 3 ovens (see " Oven requirements " on page 7)	
<input type="checkbox"/>	Fume hood (Strongly recommended: see the <i>CarrierScan™ Assay 96-Array Format Manual Workflow User Guide</i> for more information)	
<input type="checkbox"/>	One of the following thermal cyclers: <ul style="list-style-type: none"> • Applied Biosystems 9700 • Applied Biosystems Veriti™ • Applied Biosystems ProFlex™ • Eppendorf Mastercycler™ pro S (see " Thermal cyclers recommendations and protocols " on page 8)	Thermo Fisher Scientific Thermo Fisher Scientific Thermo Fisher Scientific Eppendorf
<input type="checkbox"/>	Plate shakers: (see " Plate shaker recommendations " on page 10)	

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, if you are using the three-hour precipitation workflow, or if you are running the three plate/week manual target preparation workflow.

- If you are running individual plates using the standard overnight precipitation, you will need two ovens for the workflow.
- If you are running individual plates using the three-hour precipitation workflow, a third oven is highly recommended.
- If you are running the three plate/week workflow using either overnight or three-hour precipitation, a third oven is highly recommended.

Refer to the a multi-plate workflow chapter (Chapter 6 or 7) in the *CarrierScan 96-Array Format Manual Workflow User Guide* (Pub. No. 703481) for more information.

Table 3 Suggested settings for ovens when performing the three-plate workflow for manual target preparation using overnight precipitation¹

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 ²	65°C	37°C
Day 4	48°C ²	65°C	37°C
Day 5	N/A	N/A	N/A

¹ Note that the three-plate workflow using the optional three-hour precipitation step requires different oven settings. Refer to Chapter 7 in the *CarrierScan™ 96-Array Format Manual Workflow User Guide* (Pub. No. 703481) for details.

² For preheating of the 96-well metal chamber for hybridization transfer.

Table 4 Ovens required for the CarrierScan 96-Array Format Manual Workflow

✓	Equipment	Manufacturer	Cat. No.
☐	Two ovens, any combination of the following types:		
	• ED 56 Drying and Heating Chamber ¹	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
• Applied Biosystems™ GeneChip™ Hybridization 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 CarrierScan 96-Array Format Manual Workflow.

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

Table 5 Spectrophotometers

✓	Item	Supplier	Cat. No.
<input type="checkbox"/>	One of the following spectrophotometers:		
	• DTX 880 Multimode Detector, with: – Genomic Filter Slide or • SpectraMax® High throughput Microplate Spectrophotometer	Beckman Coulter Beckman Coulter Molecular Devices	987921 – detector A30184 – filter slide Plus384

Thermal cycler recommendations and protocols

The following thermal cyclers have been verified for the CarrierScan Assay 96-Array Format Manual Workflow:

- Applied Biosystems 9700 (with gold-plated 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.

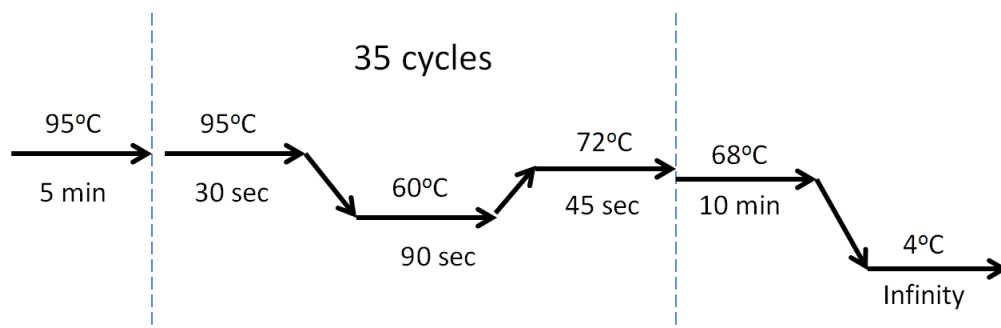


Figure 1 CarrierScan mPCR thermal cycler protocol (Stage 1A)

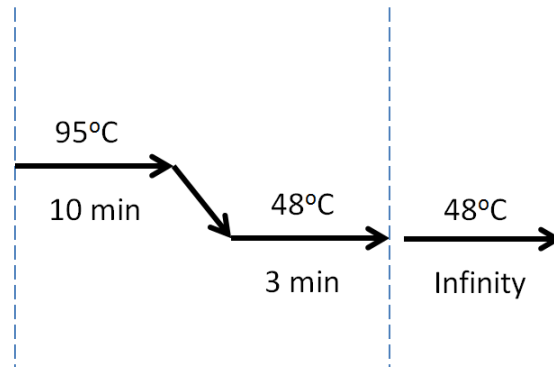



Figure 2 CarrierScan Denature thermal cycler protocol (Stage 4)

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

CarrierScan Assay 96-Array Format Manual Workflow 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.

Note: Two thermal cyclers are required if running the three plate/week manual target preparation workflow.

Thermal cycler consumables

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

Table 6 Thermal cycler consumables for the CarrierScan Assay 96-Array Format Manual Workflow

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)
Applied Biosystems Veriti	• 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)
Applied Biosystems ProFlex	• 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)
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.

Plate shaker recommendations

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

Table 7 Shakers

Shaker	Supplier	Cat. No.
Thermo Scientific™ Compact Digital Microplate Shaker	Thermo Scientific	88880023
Jitterbug™	Boekel Scientific	Model 130 000

Plate centrifuge

One plate centrifuge is required for the CarrierScan Assay 96-Array Format Manual Workflow. 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: 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 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 CarrierScan Assay 96-Array Format Manual Workflow

✓	Item	Supplier	Cat. No.
☐	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) <ul style="list-style-type: none"> – 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: <ul style="list-style-type: none"> – TX-750 high visibility swinging rotor bucket – 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

Table 9 Sample plates required for CarrierScan Assay 96-Array Format Manual Workflow

✓	Plate description	Manufacturer/ distributor	Cat. No.
<input type="checkbox"/>	One of the following PCR Plates: <ul style="list-style-type: none"> • Bio-Rad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (if using Applied Biosystems 9700, Applied Biosystems Veriti, Applied Biosystems ProFlex thermal cyclers) • Bio-Rad Hard-Shell Low Profile 96-well Full-Skirt PCR Plate (if using Eppendorf Master pro S thermal cyclers) 	Bio-Rad Bio-Rad	HSS-9641 HSP-9631 or HSP-9601
<input type="checkbox"/>	One of the following deepwell plates: <ul style="list-style-type: none"> • Eppendorf 96 Deep-well Plate, 2,000 µL (951033481) • Abgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate 	Fisher Scientific Fisher Scientific	13-864-302 AB-0932
<input type="checkbox"/>	Greiner Bio-One 96-well UV-Star Plates, 370 µL/well	Thomas Scientific VWR, Sigma-Aldrich	25801 655801

Pipettes and tips

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

Table 10 Recommended pipettes and tips for CarrierScan Assay 96-Array Format Manual Workflow

✓	Equipment	Manufacturer /Distributor	Cat. No.
<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
<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 CarrierScan Assay 96-Array Format Manual Workflow

✓	Item	Manufacturer/ distributor	Cat. No.
<input type="checkbox"/>	Microcentrifuge tubes and tube holder	MLS	various
<input type="checkbox"/>	50-mL conical centrifuge tube, polypropylene, sterile	MLS	various
<input type="checkbox"/>	15-mL conical centrifuge tube, polypropylene, sterile	MLS	various
<input type="checkbox"/>	Tube holder, 15 and 50 mL	MLS	various
<input type="checkbox"/>	96-well PCR plate rack	MLS	various
<input type="checkbox"/>	Matrix™ 25-mL reservoir	Thermo Fisher Scientific	8093-11
<input type="checkbox"/>	Solution basin, 100 mL sterile, multichannel	VWR	89092-836
<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 • Microseal 'B' Film	Thermo Fisher Scientific Bio-Rad	4306311 MSB1001
<input type="checkbox"/>	Laboratory tissue	MLS	—

Other reagents and gels required

Table 12 Other reagents required

✓	Item	Supplier	Cat. No.
<input type="checkbox"/>	QIAGEN Multiplex PCR <i>Plus</i> Kit (100)	QIAGEN	206152
<input type="checkbox"/>	Quant-iT PicoGreen dsDNA Assay Kit (recommended for DNA quantitation)	Thermo Fisher Scientific	P7589
<input type="checkbox"/>	Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Thermo Fisher Scientific	75793
<input type="checkbox"/>	2-Propanol, anhydrous, 99.5% (Isopropanol)	Sigma-Aldrich	278475

Table 13 Reagents and gels required to run QC steps

✓	Item	Supplier	Cat. No.
<input type="checkbox"/>	Mother E-Base™ Device	Thermo Fisher Scientific	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) or similar product		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"/>	E-Gel® 48 2% agarose gels (optional mPCR QC)		G8008-02
<input type="checkbox"/>	Water, nuclease-free, ultrapure MB grade		71786
<input type="checkbox"/>	50 bp DNA ladder (optional mPCR QC)	New England BioLabs Inc.	N3236S

Other lab equipment required

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

Table 14 Other equipment required

✓	Item	Supplier	Cat. No.
<input type="checkbox"/>	Freezer, -20°C	MLS	—
<input type="checkbox"/>	Refrigerator, 2°C to 8°C	MLS	—
<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	MLS	—
<input type="checkbox"/>	Markers, permanent, fine point	MLS	—
<input type="checkbox"/>	Pipet-Aid ¹	VWR	53106-220
<input type="checkbox"/>	Zerostat Anti-static Gun	Thermo Fisher Scientific	74-0014

¹ Equivalent items from other manufacturers are acceptable.

Documentation and support

Related documentation

Document	Publication number	Description
<i>CarrierScan™ Assay 96-Array Format Manual Workflow User Guide</i>	703481	This document provides instruction on running the CarrierScan Assay on 96-array format plates using a Manual Workflow and array processing on the GeneTitan MC Instrument.
<i>CarrierScan™ Assay 96-Array Format Manual Workflow Quick Reference</i>	703482	An abbreviated reference for the target preparation step of the CarrierScan Assay 96-Array Format Manual Workflow. This quick reference document is intended for experienced users.
<i>Axiom™ gDNA Sample Preparation Quick Reference</i>	MAN0017720	An abbreviated reference on preparing the genomic DNA sample.
<i>GeneTitan™ MC Protocol for Axiom™ Array Plate Processing Quick Reference</i>	MAN0017718	An abbreviated reference for processing Axiom™ Array Plates with the GeneTitan™ Multi-Channel Instrument.
<i>GeneTitan™ Multi-Channel Instrument User Guide</i>	08-0308	The GeneTitan™ Multi-Channel 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 benchtop instrument. This document details the use, care, and maintenance for the GeneTitan™ Multi-Channel Instrument.
<i>GeneTitan™ Multi-Channel Instrument Site Preparation Guide</i>	08-0305	Provides guidance on creating and maintaining the proper environment required for the GeneTitan™ Multi-Channel Instrument.

Document	Publication number	Description
Analysis and software		
<i>Reproductive Health Research Analysis Software User Guide</i>	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.
<i>Axiom™ Genotyping Solution Data Analysis Guide</i>	MAN0018363	This guide provides information and instructions for analyzing Axiom™ genotyping array data. It includes the use of Axiom™ Analysis Suite, Applied Biosystems™ Array Power Tools (formerly APT) and SNPolisher™ Package to perform quality control analysis (QC) for samples and plates, SNP filtering before downstream analysis, and advanced genotyping methods.
<i>GeneTitan™ Multi-Channel Instrument Site Preparation Guide</i>	08-0305	Provides guidance on creating and maintaining the proper environment required for the GeneTitan Multi-Channel Instrument.
<i>Applied Biosystems™ GeneChip™ Command Console (GCC) Software User Guide</i>	702569	This user guide provides instructions for 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.

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Limited product warranty

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15 October 2020

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