DANSR Kit US-RUO
P/N: 08318085001

Package Insert

For Research Use Only. Not for use in diagnostic procedures.

PROFESSIONAL USE ONLY

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PRODUCT DESCRIPTION

The Digital Analysis of Selected Regions (DANSR) Kit US-RUO is for research use in analysis of cell free DNA (cfDNA) samples.

PRINCIPLE OF THE PROCEDURE

The DANSR Kit US-RUO requires cfDNA that has been isolated using a commercially available cfDNA extraction kit from approximately 4mL of plasma collected using a cell-free DNA collection tube (Roche PN 07785674001 or equivalent).

The DANSR Kit US-RUO (P/N 08318085001) in combination with the Ariosa cell-free DNA System (AcfS) Software US-RUO (P/N 08012300001), including the FORTE_R.DLL algorithm used for analysis, allows for testing of defined chromosomal conditions. The AcfS test is designed to be used with a set of required equipment and AcfS Software, collectively termed as the Ariosa cell-free DNA System.

The AcfS test enables execution of 3 sequential processes on sets of 48-96 specimens which includes 48-95 cell free DNA (cfDNA) specimens and 1 Assay Performance Control (APC). First, the DANSR Kit US-RUO utilizes the DANSR assay process to amplify a set of universal polymerase chain reaction (UPCR) products from genomic intervals on chromosomes 1-12, 13, 18, 21, 22, X, and Y in each sample, and to quantify each UPCR product in each sample by hybridization to a custom oligonucleotide microarray. Next, the FORTE_R.DLL algorithm evaluates the microarray fluorescence intensity data to calculate the probabilities of trisomy 13, trisomy 18, and trisomy 21, 22q11.2 deletion; and sex chromosomes aneuploidy (Monosomy X, XXX, XXY, XYY, XXYY); and to determine fetal sex in each specimen. See also *FSD-000108 AcfS Software US-RUO Package Insert*.

DANSR Assay Targeted Amplification

A targeted amplification process termed Digital Analysis of Selected Regions[2,3,4] (DANSR) is used to simultaneously amplify UPCR products corresponding to approximately 7,000 genomic intervals across the chromosomes of interest from each of the cfDNA specimens.

The specimens are first purified to eliminate potential impurities within the cfDNA. The purified cfDNA samples are then denatured to expose 3'OH ends for biotinylation, and then the Terminal deoxy Transferase (TdT) is used to biotinylate the 3'OH ends of the single stranded cfDNAs.

Trios of DANSR oligonucleotides targeting specific genomic loci are then annealed to the biotinylated cfDNA to form cfDNA-DANSR oligonucleotide complexes. The cfDNA-DANSR oligonucleotide complexes are then captured onto streptavidin (SA)-coated magnetic beads and washed. Next, the annealed DANSR oligonucleotides are ligated to form the DANSR assays. The left and right ends of all DANSR assays contain 5' and 3' extensions, respectively, corresponding to universal PCR (UPCR) primer binding sites. The ligated DANSR assay products are eluted from the SA-beads and used to inoculate UPCR reactions. Thermal cycling of the UPCR reactions is performed in a post-PCR laboratory and yields DANSR assay UPCR products.

The DANSR assay targeted amplification process is performed using a Roche configured Library Robot (Roche P/N: 07759371001) to execute specimen and reagent manipulation and barcode scanning. Reaction incubations are performed using a Bio-Rad C1000 thermal cycler.

Microarray-Based Quantification of DANSR Assay UPCR Products

The DANSR assay UPCR products are quantified using identical copies of a custom oligonucleotide microarray configured on an Array Of Pegs (AOP) to enable simultaneous processing[3,4]. Each microarray contains 16 redundant copies of approximately 7,000 unique features, where each feature consists of a specific oligonucleotide sequence.

The DANSR assay UPCR products undergo an additional round of amplification and are then purified to create high-concentration DANSR assay UPCR products. The DANSR assay UPCR products are digested resulting in a set of DANSR assay microarray targets that contain a unique sequence complimentary to one of the sequences on the microarray. The DANSR assay digested UPCR products are then hybridized to microarrays on a single AOP. The AOP is washed to remove excess target, labeled using a fluorescently conjugated oligonucleotide, and washed again. The AOP is imaged using a Concerto Imager to quantify the fluorescence signal corresponding to each feature for each sample on the AOP. The Concerto Imager generates a file for each array containing the fluorescence intensity values for each feature on the array.

The microarray-based DANSR assay quantification process is performed by a Roche configured Detection Robot (Roche P/N: 07759363001) to execute specimen, reagent, and array manipulation and barcode scanning. Reaction incubations are performed using a Bio-Rad C1000 thermal cycler. The array hybridization is performed using a Binder incubator. Array imaging is performed using a Concerto Imager.

FORTE Algorithm Analysis

The FORTE[3,4,5] algorithm is used to analyze the fluorescence intensity data from the 48-96 unique biological samples. The FORTE algorithm aggregates data from the features for each DANSR assay to obtain a robust median intensity for each DANSR assay in each sample. The FORTE algorithm then normalizes the relative intensities of the DANSR assays to eliminate systematic sample, locus, and allele biases. The FORTE algorithm next evaluates the relative intensities of DANSR assays corresponding to the two alleles of each polymorphic locus to estimate the allele frequency of the polymorphic locus in each sample. The FORTE algorithm then identifies loci that are informative for estimating fetal fraction in each sample (i.e., loci where the maternal genotype is homozygous for one allele, and the fetus has inherited a different allele), and uses the allele frequencies of these informative loci to estimate the fraction of fetal DNA in each sample. The FORTE algorithm next evaluates the relative intensities of DANSR assays corresponding to non-polymorphic loci to estimate the relative concentration of each of chromosomes 13, 18, 21, X and Y, as well as the 22q11.2 chromosomal region. The FORTE algorithm next assesses the probability of trisomy of chromosomes 13, 18 and 21, 22q11.2 deletion, and sex chromosome aneuploidy (monosomy X, XXX, XXY, XYY, XXYY), by computing the relative likelihood of obtaining the observed chromosome concentration and fetal fraction data from an aneuploid sample versus from a euploid sample. The FORTE algorithm adjusts the raw probability scores for each sample for the prior probability associated with the maternal age and gestational age of the sample. The FORTE algorithm then caps these adjusted probability scores at 0.01% and 99%. For trisomy and sex chromosome aneuploidy, the FORTE algorithm classifies capped probability scores <1% as low probability, and probability scores of ≥1% as high probability. For 22q11.2, the FORTE algorithm classifies probability scores of <1% as no evidence of a deletion observed, and probability scores of ≥1% as high probability of a deletion. In addition, the FORTE algorithm also evaluates the relative intensities of chromosome Y loci to determine fetal sex.

MATERIALS PROVIDED

The test consists of two components: the DANSR Kit US-RUO (P/N 08318085001) and the Ariosa cell-free DNA System (AcfS) Software US-RUO (P/N 08012300001), including the FORTE_R.DLL algorithm. The DANSR Kit US-RUO includes reagents and materials to enable analysis of 8 sets of runs with a batch size of 48-95 plasma-derived cfDNA samples plus one assay performance control (APC). Table 1 lists the components of the DANSR Kit US-RUO, and Tables 2 through 7 list the contents of each component kit.

NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples

Table 1: DANSR Kit US-RUO Components

Name	Maximum Number of Tests	Roche P/N	Shipping
DANSR LIBRARY BOX 1	8 X 96	07759207001	Ambient
DANSR LIBRARY BOX 2	8 X 96	07759240001	Dry Ice
DANSR DETECTION BOX 1	8 X 96	07759215001	Ambient
Array 96s384 BOX 2	8 X 96	07871643001	Ambient
DANSR DETECTION BOX 3	8 X 96	07759231001	Ambient
DANSR DETECTION BOX 4	8 X 96	07759258001	Dry Ice

Table 2: DANSR LIBRARY BOX 1 (P/N 07759207001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
AM1T	Ariosa Magnetospheres 1	250 mL Bottle	150 mL	1	2 to 8°C Library Lab	07871473001
AM2T	Ariosa Magnetospheres 2	250 mL Bottle	200 mL	1	2 to 8°C Library Lab	07871503001
AM3T	Ariosa Magnetospheres 3	250 mL Bottle	200 mL	1	2 to 8°C Library Lab	07871511001
R2B	Resuspend 2 Buffer	250 mL Bottle	175 mL	1	2 to 8°C Library Lab	07871520001
LEB	Ligate Equilibrate Buffer	500 mL Bottle	300 mL	1	2 to 8°C Library Lab	07871538001
R4B2	Resuspend 4 Buffer 2	250 mL Bottle	200 mL	1	2 to 8°C Library Lab	07871546001
#NAP	Nucleic Acid Plate Barcode	Barcode	N/A	8	20 to 25°C Library Lab	08798800001
#UNA	96-well UNA Plate Barcode	Barcode	N/A	8	20 to 25°C Library Lab	08798818001
#AM1	AM1 Plate Barcode	Barcode	N/A	8	20 to 25°C Library Lab	08798826001
#AM2	AM2 Plate Barcode	Barcode	N/A	8	20 to 25°C Library Lab	08798834001
#AM3	AM3 Plate Barcode	Barcode	N/A	8	20 to 25°C Library Lab	08798842001

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
#TCP	Thermal Cycle Plate Barcode	Barcode	N/A	8	20 to 25°C Library Lab	08798893001
#PPP	Purify & Prepare Plate Barcode	Barcode	N/A	8	20 to 25°C Library Lab	08798907001
#ETH	Ethanol Barcode	Barcode	N/A	2	20 to 25°C Library Lab	08798958001
#TD-AM1T	Ariosa Magnetospheres 1 Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08798869001
#TD-AM2T	Ariosa Magnetospheres 2 Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08798877001
#TD-AM3T	Ariosa Magnetospheres 3 Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08798885001
#TD-ETH	Ethanol Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08798966001
#TD-R2B	Resuspend 4 Buffer 2 Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08798974001
#TD-LEB	Ligate Equilibrate Buffer Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08798982001
#TD-LWB	Ligate Wash Buffer Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08799008001
#TD-R4B2	Resuspend 4 Buffer 2 Trough Barcode	Barcode	N/A	16	20 to 25°C Library Lab	08799016001

# indicates barcode labels

*GHS08 Health Hazard Symbol: H360 'May damage fertility or the unborn child' applies to the hazardous material formamide, which is included in ADB3 and LBB buffers. Refer to DANSR Library Box 2 Safety Data Sheet for appropriate formamide-specific hazardous material handling.

Table 3: DANSR LIBRARY BOX 2 (P/N 07759240001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
PPPT	Purify and Prepare Plate	50 mL Tube	18 mL	1	-30 to -15°C Library Lab	07871651001
TCPT	Thermal Cycle Plate	50 mL Tube	36 mL	1	-30 to -15°C Library Lab	07871660001
BTM	BioTinylate Master Mix	15 mL Tube	1.7 mL	8	-30 to -15°C Library Lab	07871678001
ADB3*	Anneal DNA Buffer 3*	15 mL Tube	6 mL	8	-30 to -15°C Library Lab	07871686001
LBB*	Ligate Bead Buffer*	15 mL Tube	5.1 mL	8	-30 to -15°C Library Lab	07871694001
LIM	Ligate Master Mix	15 mL Tube	6 mL	8	-30 to -15°C Library Lab	08798656001

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
LWB	Ligate Wash Buffer	250mL Bottle	200 mL	1	-30 to -15°C Library Lab	07871716001
APC	Assay Performance Control (Euploid)	10mL Tube	1.9 mL	1	-30 to -15°C Library Lab	08798761001

Table 4: DANSR DETECTION BOX 1 (P/N 07759215001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
AIB	Array Imaging Buffer	250 mL Bottle	225 mL	1	2 to 8°C Detection Lab	07871562001
PHB	Post Hyb Wash Buffer	500 mL Bottle	350 mL	1	2 to 8°C Detection Lab	07871597001
PLB	Post Label Wash Buffer	500 mL Bottle	350 mL	1	2 to 8°C Detection Lab	07871619001
R7B2	Resuspend 7 Buffer 2	250 mL Bottle	175 mL	1	2 to 8°C Detection Lab	07871554001

Table 5: Array 96S384 Box 2 (P/N 07871643001) Contents

Component	Name	Vessel	Quantity	Storage	Roche P/N
AOP 96	Array of Pegs	Array	8	2 to 8°C Detection Lab	07955618001

Table 6: DANSR DETECTION BOX 3 (P/N 07759231001) Contents

Component	Name	Vessel	Quantity	Storage	Roche P/N
BT	Blue Tray	Plastic	4	20 to 25°C Detection Lab	07871759001
GSK	Gaskets	Rubber	10	20 to 25°C Detection Lab	07871767001
PST	Plate Scan Tray	Plastic	8	20 to 25°C Detection Lab	07871732001
ST	Stain Tray	Plastic	8	20 to 25°C Detection Lab	07871724001
#ETH	Ethanol Barcode	Barcode	2	20 to 25°C Detection Lab	08798958001
#ALM	Array Label Mix Barcode	Barcode	10	20 to 25°C Detection Lab	08798940001
#PHB	Post Hyb Buffer Barcode	Barcode	4	20 to 25°C Detection Lab	08798923001
#PLB	Post Label Buffer Barcode	Barcode	4	20 to 25°C Detection Lab	08798931001
#TD-R7B2	Resuspend 7 Buffer 2 Trough Barcode	Barcode	16	20 to 25°C Detection Lab	08798915001

Component	Name	Vessel	Quantity	Storage	Roche P/N
#TD-ETH	Ethanol Trough Barcode	Barcode	16	20 to 25°C Detection Lab	08798966001
#TD-UPM2	Universal Primer Mix 2 Trough Barcode	Barcode	16	20 to 25°C Detection Lab	08799024001
#TD-HAB	Hyb Anneal Buffer Trough Barcode	Barcode	16	20 to 25°C Detection Lab	08799032001
#TD-AIB	Array Imaging Buffer Trough Barcode	Barcode	16	20 to 25°C Detection Lab	08799105001

# indicates barcode labels

Table 7: DANSR DETECTION BOX 4 (P/N 07759258001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
ALM	Array Label Mix	50 mL Tube	22 mL	8	-30 to -15°C Detection Lab	07871775001
UPM2	Universal PCR Mix 2	15 mL Tube	13 mL	8	-30 to -15°C Detection Lab	07871791001
HAB	Hyb Anneal Buffer	250 mL Bottle	140 mL	1	-30 to -15°C Detection Lab	07871805001

MATERIALS AND ACCESSORIES REQUIRED

The DANSR Kit US-RUO is designed to be used with a set of required equipment and software, collectively termed the Ariosa cell-free DNA System (AcfS). Tables 8 through 10 list the components of the AcfS.

Table 8: AcfS Equipment

Equipment	Source	P/N	Quantity
Concerto Imager	Roche (F. Hoffmann-La Roche, Ltd.)	120V: 08088900001 or 09337407001	1
Concerto Imager Workstation	Roche (F. Hoffmann-La Roche, Ltd.)	08051844001	1
Library Robot, with Workstation, Monitor and Power Cord	Roche (F. Hoffmann-La Roche, Ltd.)	Library Robot: 07759371001 Workstation: 08464103001 or 09121633001; Monitor: 07871899001; Power Cord:07759568001	1
Detection Robot, with Workstation, Monitor and Power Cord	Roche (F. Hoffmann-La Roche, Ltd.)	Detection Robot: 07759363001 Workstation: 08464103001 or 09121633001; Monitor: 07871899001; Power Cord:07759568001	1
AcfS Analysis Server	Roche (F. Hoffmann-La Roche, Ltd.)	07759282001 or 09121641001	1
AcfS L&D Equipment Installation Bundle	Roche (F. Hoffmann-La Roche, Ltd.)	07759274001	1
Binder KB53 Incubator, with Big Bear 1mm throw Plate Shaker	Roche (F. Hoffmann-La Roche, Ltd.)	120V: 08041652001 or 08478074001	1

Table 9: AcfS Software, including FORTE_R.DLL (P/N 08012300001)

Software	Source
Director	Roche Sequencing Solutions, Inc.
Analysis Service	Roche Sequencing Solutions, Inc.
FORTE_R.DLL	Roche Sequencing Solutions, Inc.
Re-Analysis Service	Roche Sequencing Solutions, Inc.
Report Generator	Roche Sequencing Solutions, Inc.

Table 10: AcfS User Guide

Document	Source	Publication
AcfS User Guide	Roche Sequencing Solutions, Inc.	1200000414595

Table 11: Materials required but not provided

Item	Source	P/N	# per kit
Thermal Cycler C1000	Bio-Rad	185-1197	1 (2 required)
Centrifuge 5810 with microplate rotor*	Eppendorf	5810000068, 53513-872	1 (2 required)
EDTA, disodium, 0.01M*	VWR	BDH7621-1	1 L
Ethanol, 70%*	Teknova	E0030	850 mL
TWEEN 80, 100%*	Sigma	P5188-100ML	100 mL
PCR plate, hard shell, 0.2mL, 96-well	Bio-Rad	HSP-9601	24
Storage plate, square well, 2.2 mL, 96-well	Thermo Fisher	AB-0932	24
Storage plate, 0.8 mL, 96-well	Thermo Fisher	AB-0765	8
Disposable trough, 100 mL	Tecan	10613048	9
Disposable trough, 320 mL	VWR	25608-904	3
Adhesive plate seal*	Bio-Rad	MSB-1001	100
DNA LoBind Tube, 1.5mL*	Eppendorf	1130 108.051	8
Pipette, single-channel, L-200XLS+*	Rainin	17014391	1
Pipette tip, 200 µL, filter, sterile*	Rainin	17014963	672

* Or equivalent.

SAFETY INFORMATION

Safety Data Sheets (SDSs) for the DANSR Kit US-RUO can be obtained from https://dialog1.roche.com/ using the Roche part numbers listed in Table 1.

Handle and dispose of DANSR Kit US-RUO reagents according to the SDSs. Appropriate precautions (including use of personal protective equipment (PPE) should be used when handling and disposing of DANSR Kit US-RUO reagents.

Blood and plasma specimens should be considered potentially infectious material. Appropriate precautions (including use of PPE) should be used when handling potentially infectious specimens.

Appropriate precautions (including use of personal protective equipment (PPE)) should be used with automated instrumentation and magnetic equipment. Operate automated instrumentation according to the manufacturer's instructions and the AcfS User Guide. .

STORAGE AND HANDLING REQUIREMENTS

Upon receipt of the DANSR Kit US-RUO, verify the contents of the kit using the MATERIALS PROVIDED tables above. If any DANSR Kit US-RUO component is missing, contact your Roche Field Applications Specialist.

DANSR Library Box 2 and DANSR Detection Box 4 are shipped on dry ice. If there is no residual dry ice in the outer shipping container, or if the outer shipping container has been opened during shipping, contact your Roche Field Applications Specialist.

Store the DANSR Kit US-RUO components at recommended temperatures and locations as indicated in the MATERIALS PROVIDED tables above.

Expiration dates can be found on the reagents within the kit. Do not use reagents after the expiration date.

NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples.

AM1T, AM2T, AM3T, TCPT, and PPPT are each provided in one bulk container and should be stored in the Library Lab. These reagents must be dispensed into eight 96 well plates each (termed AM1, AM2, AM3, TCP, and PPP plates, respectively) prior to use, according to the Reagent Preparation Section of this package insert. Dispensed AM1, AM2, and AM3 plates have the same expiration date of their respective bulk container configurations when stored at 4ºC (2 to 8°C). Dispensed AM1 and AM2 should be stored in the Library Lab, and dispensed AM3 should be stored in the Detection Lab. Dispensed TCP and PPP plates have the same expiration date of their respective bulk container configurations when stored at -20ºC (-30 to -15°C).
Dispensed TCP should be stored in the Library Lab and dispensed PPP should be stored in the Detection Lab.

BTM, ADB3, LBB, LIM, ALM, and UPM2 are each provided as eight single use aliquots, where each aliquot contains sufficient material to process one batch of 48-95 samples and 1 APC. Do not reuse residual reagents in single use aliquots. If stored at recommended temperatures and if used within 6 hours of removal from storage to 20 to 25°C, these reagents may be used until the expiration date stated on their respective labels.

R2B, LEB, R4B2, LWB, AIB, PHB, PLB, and R7B2 are each provided in one bulk container containing sufficient volumes to enable processing of eight batches of 48-95 samples and 1 APC. If stored at recommended temperatures when not in use, used within 6 hours of removal from storage to 20 to 25°C, and subsequently returned to recommended storage temperatures, these reagents may be used for eight such cycles until the expiration date stated on their respective labels.

HAB is provided in one bulk container containing sufficient volumes to enable processing of eight batches of 48-95 samples and 1 APC. If stored at -20°C(-30 to -15ºC) prior to use, used within 6 hours of removal from storage to 20 to 25°C, and subsequently returned to 4°C (2 to 8ºC), this reagent may be used for eight cycles until the expiration date stated on its label.

The APC is provided in one bulk container, and should be dispensed into eight individual aliquots each prior to storage in order to avoid multiple freeze-thaw cycles, according to the Reagent Preparation Section of the package insert. Aliquoted APC has the same expiration date of its respective bulk configuration when stored at -20ºC (-30 to -15°C) and should be stored in the Library Lab.

SPECIMEN COLLECTION, TRANSPORT, STORAGE, AND PREPARATION

1. It is recommended that two (2) tubes of maternal whole blood* are collected by venipuncture using the Roche Cell-Free DNA collection tube (P/N 0783188900 or equivalent), according to the manufacturer's instructions. Transport and store the whole blood specimen according to the cfDNA-compatible blood collection tube manufacturer's instructions.

2. Separate maternal plasma by centrifuging the whole blood specimen at ambient temperature at 1,600 rcf (g) for 10 to 15 minutes, with minimum acceleration and deceleration. After centrifugation, carefully decap the blood tube and remove the top plasma layer to an appropriate container, taking care to avoid the opaque buffy coat layer which contains the maternal blood cells. Additional centrifugation of the separated plasma may be performed but is not required if the preceding step is done appropriately. Blood specimens collected in the Roche Cell-Free DNA tubes are stable for up to 7 days from draw if stored between 18 and 25°C. Plasma specimens transferred from the blood collection tube are stable for 5 days from transfer if stored between 20 and 25ºC and up to 3 years if stored at -30 to -15°C.

3. Isolate cfDNA from approximately 4mL maternal plasma using a commercially available DNA isolation kit or established in-house procedure that has been validated for use in extracting cfDNA from plasma according to the manufacturer's instructions. The QiaSymphony SP/AS nucleic acid extraction platform, Magna Pure 24 platform, and MagnaPure 96 platform have been validated for use with the test on AcfS.[1] Use the same cfDNA isolation biochemistry on all specimens to be processed together in a batch. Elute the cfDNA specimen to a final volume of 150µL of elution buffer. For optimal AcfS performance, it is recommended to not store DNA following DNA extraction and to proceed immediately to the "Create UNA task".

*See Quality Review and Reporting of Results section for more information on use of the second tube.

INSTRUCTIONS FOR USE

Professional Use Only

Reagent Preparation

1. Refer to the Ariosa cell-free DNA System User Guide (1200000414595) for detailed guidance on executing the reagent preparation tasks.

2. The DANSR Kit US-RUO is configured to enable analysis of 8 sets of 48-96 specimens, including 48-95 plasma-derived cfDNA specimens and 1 APC. Reagent preparation tasks 3-7 of this section accomplish dispensing of 5 reagents (AM1T, AM2T, AM3T, TCPT, and PPPT) that are shipped in bulk form into eight 96 well plates formatted for use in Director tasks executed by the Library Robot and the Detection Robot. Reagent preparation task 8 of this section accomplishes aliquoting the bulk APC reagent into eight individual 1.5mL tubes for storage purposes.

3. Label eight 2.2mL 96 deep well plates (Thermo Fisher P/N AB-0932) with the AM1 barcodes provided with the kit. Execute the Director “Prep AM1” task on the Library Robot to dispense bulk AM1T reagent into 8 AM1 plates. The robot dispenses 98µL AM1T into each well of each of the 8 AM1 plates. Upon task completion, seal the AM1 plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at 4°C (2 to 8ºC) in the Library Lab. Dispensed AM1 plates have the same expiration date as bulk AM1T when stored at 4°C (2 to 8ºC).

4. Label eight 2.2mL 96 deep well plates (Thermo Fisher P/N AB-0932) with the AM2 barcodes provided with the kit. Execute the Director “Prep AM2” task on the Library Robot to dispense bulk AM2Treagent into 8 AM2 plates. The robot dispenses 144µL AM2T into each well of each of the 8 AM2 plates. Upon task completion, seal the AM2 plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at 4°C (2 to 8ºC) in the Library Lab. Dispensed AM2 plates have the same expiration date as bulk AM2T when stored at 4°C (2 to 8ºC).

5. Label eight 2.2 mL 96 deep well plates (Thermo Fisher P/N AB-0932) with the AM3 barcodes provided with the kit. Execute the Director “Prep AM3” task on the Library Robot to dispense bulk AM3T reagent into 8 AM3 plates. The robot dispenses 144µL AM3T into each well of each of the 8 AM3 plates. Upon task completion, seal the AM3 plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at 4°C in the Detection Lab. Dispensed AM3 plates have the same expiration date as bulk AM3T when stored at 4°C (2 to 8ºC).

6. Label eight 96 well hard shell PCR plates (Bio-Rad P/N HSP-9601) with the TCP barcodes provided with the kit. Execute the Director “Prep TCP” task on the Library Robot to dispense bulk TCPT reagent into the 8 TCP plates. The robot dispenses 27µL TCPT into each well of each of the eight TCP plates. Upon task completion, seal the TCP plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at -20°C (-30 to -15ºC) in the Library Lab. Dispensed TCP plates have the same expiration date as bulk TCPT when stored at -20°C (-30 to -15ºC).

7. Label eight 96 well hard shell PCR plates (Bio-Rad P/N HSP-9601) with the PPP barcodes provided with the kit. Execute the Director “Prep PPP” task on the Library Robot to dispense bulk PPPT reagent into the 8 PPP plates. The robot dispenses 11µL PPPT into each well of each of the eight PPP plates. Upon task completion, seal the PPP plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at -20°C (-30 to -15ºC) in the Detection Lab. Dispensed PPP plates have the same expiration date as bulk PPPT when stored at -20°C (-30 to -15ºC).

8. Aliquot the APC into eight labeled 1.5mL tubes (Eppendorf P/N 1130 108.051) with the APC, lot number, and expiration date. Use a Rainin L-200XLS+ pipette (P/N 17014392) and sterile filtered pipette tips (Rainin P/N 17014963) to aliquot 200µL of the APC into its corresponding eight 1.5mL tubes. Upon task completion, cap the 1.5mL tubes, and store the 1.5mL tubes at -20°C (-30 to -15ºC) in the Library Lab. The APC aliquots have the same expiration date as the bulk APC when stored at -20°C (-30 to -15ºC) and should be used with the same lot of reagents provided in the DANSR Library Box 2 (P/N 07759240001).

DANSR Assay Targeted Amplification

1. Refer to the Ariosa cell-free DNA System User Guide (1200000414595) for detailed instructions on executing the DANSR assay targeted amplification process using the Library Robot.

2. Obtain the 48-95 subject-derived cfDNA specimens and the APC to be processed together as a batch through the DANSR Kit US-RUO. Label a 96-well 800µL/well plate (ThermoFisher P/N AB-0765) with a Nucleic Acid Plate (NAP) barcode. If the samples are not already eluted in the 96-well plate via an automated method, use a pipette (Rainin L-200XLS+, P/N 17014392) and sterile filtered pipette tips (Rainin P/N 17014963) to transfer 150µL of each of 48-95 subject-derived cfDNA specimens and 150µL of the APC into separate wells of the NAP plate, being careful to change tips between transfers..

3. If running less than 96 samples, load samples in order from top to bottom then left to right, so that columns are filled before rows (fill A1-H1 before A2). See figure below for sample loading order

4. Note the destination well for each sample including the APC. Seal the NAP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and proceed immediately to the Create UNA task (step 8 below).

5. Create a NAP File (also termed a Specimen Sample Sheet) containing the information regarding the samples in the NAP plate. The required information is listed in table 13.

   NOTE: The NAP File needs to have a minimum of 48 cfDNA unique specimens from plasma of pregnant women denoted as “Specimen”. The assay performance control is not considered a unique specimen and should be labeled as “Control”.

6. The NAP File is a tab-delimited file consisting of a Column Header row, followed by one row per sample for each of the 48-96 samples in the NAP plate (with row 1 for the sample in well A1, row 2 for well B1, row 3 for well C1, etc.) as demonstrated in Figure 1. .

7. The barcode of the NAP plate (AD#-xxxxxxxx-NAP) must exactly match the name of the NAP file. For example, the file name for barcoded plate AD12345678-NAP must be AD12345678-NAP.txt. Mismatched barcodes and file names will cause task failures. The file format must be a tab delimited text (.tab or .txt).

	1	2	3	4	5	6	7	8	9	10	11	12
A	**1**	**9**	**17**	**25**	**33**	**41**	**49**	**57**	**65**	**73**	**81**	**89**
B	**2**	**10**	**18**	**26**	**34**	**42**	**50**	**58**	**66**	**74**	**82**	**90**
C	**3**	**11**	**19**	**27**	**35**	**43**	**51**	**59**	**67**	**75**	**83**	**91**
D	**4**	**12**	**20**	**28**	**36**	**44**	**52**	**60**	**68**	**76**	**84**	**92**
E	**5**	**13**	**21**	**29**	**37**	**45**	**53**	**61**	**69**	**77**	**85**	**93**
F	**6**	**14**	**22**	**30**	**38**	**46**	**54**	**62**	**70**	**78**	**86**	**94**
G	**7**	**15**	**23**	**31**	**39**	**47**	**55**	**63**	**71**	**79**	**87**	**95**
H	**8**	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**

Figure 1: Sample Identification in a NAP File

Table 12: NAP FileContents

Column Header	Description	Accepted Values
HL_SpecimenID	A unique identifier for the sample.	Alphanumeric string excluding the following characters: <>:"/|\?*
HL_MaternalAge_Years	Maternal age in years at the expected delivery date.	10-99
HL_GestationalAge_Weeks	Gestational age of the fetus in whole weeks.	10-40
HL_GestationalAge_Days	Any additional days that were not included in GestationalAge_Weeks of the fetus.	0-6
HL_IVFStatus	Indication of egg donor source.	Self Nonself No
HL_EggDonorAge_Years	If applicable, age of the mother providing the donor egg at the time of egg donation.	Empty or 12-76
HL_TestId	The test ordered for the sample.	Trisomy Trisomy + SCAP Trisomy+ MX Trisomy+ FS Trisomy+ FS + SCAP Trisomy+ FS + MX Trisomy+ 22q Trisomy+ SCAP + 22q Trisomy+ MX + 22q Trisomy+ FS + 22q Trisomy + FS + SCAP + 22q Trisomy + FS + MX + 22q
HL_NumberOfFetus	The number of fetuses present in the sample.	1 or 2
HL_SampleType	Identifies sample as Specimen or APC.	Specimen or Control

8. Execute the Director “Accession Sample Sheet” task on the Library Robot to accession the NAP File. Director saves the NAP File to the Analysis Server. Director subsequently appends process data (reagent container barcodes, timestamps, etc.) to this file as the samples proceeds through the test process. Ultimately, the file will contain the history for the run of the test process performed on the Robots and Imager and will be used as input to the analysis process of the FORTE algorithm.

   NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples.

9. Execute the Director “Create UNA” task on the Library Robot to purify the DNA samples in the NAP plate. In the Create UNA task, the robot transfers 140µL from each well of the NAP plate to the corresponding well of an AM1 plate containing 98µL of a paramagnetic bead-containing solution (AM1). (For batches of less than 96 samples, the robot will still do the transfer task even without samples in the well.) After incubating the AM1 plate for 5 minutes with mixing at 2000rpm and 5 minutes without mixing, the robot incubates the AM1 plate for 10 minutes on a neodymium magnet to immobilize the AM1 beads near the bottom of the wells. The robot then transfers the 238µL cfDNA-containing supernatant from each well of the AM1 plate to the corresponding well of an AM2 plate containing 144µL of a second paramagnetic bead-containing solution (AM2). After incubating the AM2 plate for 5 minutes with mixing at 2000rpm and 5 minutes without mixing, the robot incubates the AM2 plate for 20 minutes on a neodymium magnet to immobilize the AM2 beads near the bottom of the wells. After removing the supernatant from each well of the AM2 plate, the robot washes the AM2 beads with 200µL 70% EtOH (ETH), and then elutes cfDNA from the beads by dispensing 25µL Resuspension 2 Buffer (R2B) to each well. After incubating the AM2 plate for 5 minutes with mixing at 1200rpm and 5 minutes without mixing, the robot incubates the AM2 plate on a magnet for 2 minutes to immobilize the AM2 beads near the bottom of the wells, and transfers the cfDNA-containing R2B supernatant to a new 96-well hard shell PCR plate (Bio-Rad P/N HSP-9601) labeled with a Unified Nucleic Acid (UNA) barcode.

10. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate at 95°C for3 minutes using the C1000 Thermal Cycler program Inc1_UNA to denature the DNA in preparation for biotinylation. After denaturation, proceed to the “Biotinylate UNA” task in Director or store the UNA plate at -20°C (-30 to -15ºC) for up to 3 days.

11. Remove the UNA plate from the thermal cycler or from -20°C storage and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion. Avoid shaking or lifting the plate. Execute the Director “Biotinylate UNA” task on the Library Robot to initiate biotinylation of the cfDNA samples in the UNA plate. In the Biotinylate UNA task, the robot dispenses 8µL of BioTinylation Master mix (BTM) to each well in the UNA plate and then shakes the plate at 1900 rpm for 1 minute.

12. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate at 37°C for 1 hour using the C1000 Thermal Cycler program Inc2_UNA to execute the biotinylation reaction. Remove the UNA plate from the thermal cycler and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion to minimize splashing or carryover between wells. Avoid shaking or lifting the plate.

13. Execute the Director “Anneal UNA” task on the Library Robot to anneal the DANSR assay oligonucleotides to the DNA samples in the UNA plate. In the Anneal UNA task, the robot dispenses 40µL of Anneal DNA Buffer 3 (ADB3) containing the DANSR assay oligonucleotides to each well in the UNA plate and then shakes the plate at 1600 rpm for 1 minute.

14. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate while gradually decreasing the temperature from 70 to 30°C over the course of 2 hours using the C1000 Thermal Cycler program Inc3_UNA to execute the annealing process. After the 2 hour ramp-down, hold the UNA plate for at least 45 minutes at 30°C before removing it from the thermocycler. Remove the UNA plate from the thermal cycler and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion to minimize splashing or carryover between wells. Avoid shaking or lifting the plate.

15. Execute the Director "Ligate UNA" task on the Library Robot to ligate the annealed DANSR oligonucleotides. In the Ligate UNA task, the robot dispenses 30µL of Ligate Bead Buffer (LBB) containing streptavidin (SA)-coated paramagnetic beads to each well of the UNA plate and then shakes the plate for 1 minute at 1400 rpm. The robot then incubates the UNA plate at 30°C for 30 minutes to allow the biotinylated cfDNA-DANSR oligonucleotide complexes to bind to the SA-beads. The robot then incubates the UNA plate for 2 minutes on a neodymium magnet to immobilize the SA-beads near the bottom of the wells. After removing the supernatant from each well of the UNA plate, the robot washes the SA-bound cfDNA-DANSR oligonucleotide complexes twice with 50µL of Ligate Wash Buffer (LWB). The robot dispenses the LWB to each well, shakes the plate at 1900 rpm for 1 minute, incubates the plate on a neodymium magnet for 2 minutes, removes the supernatant, and repeats this process for a total of two washes. The robot then washes the SA- bound cfDNA-DANSR oligonucleotide complexes twice with 50µL of Ligate Equilibrate Buffer (LEB) by dispensing the buffer to each well, shaking the plate at 1900 rpm for 1 minute, incubating the plate on a neodymium magnet for 2 minutes, removing the supernatant, and repeating this process. Next, the robot ligates the annealed DANSR oligonucleotide by dispensing 37µL of Ligate Master Mix (LIM) to each well of the UNA plate, shaking the plate at 2000 rpm for 10 seconds, incubating the plate at 50°C for 10 minutes, forming the ligated DANSR assays The LIM supernatant is then removed by incubating the plate on a neodymium magnet to immobilize the SA-bound cfDNA-DANSR assay complexes near the bottom of the wells and remove the LIM supernatant from each well. The robot then washes the SA--bound cfDNA-DANSR assay complexes twice with 50µL LEB by dispensing the buffer to each well, shaking the plate at 1900 rpm for 1 minute, incubating the plate on a neodymium magnet for 2 minutes, removing the supernatant, and repeating this process. Finally, the robot dispenses 30µL Resuspend 4 Buffer 2 (R4B2) to each well in the UNA plate and shakes the plate at 1900 rpm for 1 minute.

16. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate at 95°C for 1 minute using the C1000 Thermal Cycler program Inc4_UNA to denature the ligated DANSR assays from the SA-bead immobilized DNA. Remove the UNA plate from the thermal cycler and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion. Avoid shaking or lifting the plate.

17. Execute the Director "Inoc TCP" task on the Library Robot to transfer the ligated DANSR assays from the UNA plate to a Thermal Cycle Plate (TCP) containing a universal PCR master mix. In the "Inoc TCP" task, the robot incubates the UNA on a magnet for two minutes, transfers 25µL of supernatant containing the eluted, ligated DANSR assays from each well of the UNA plate to the corresponding well of the TCP plate, and shakes the TCP plate at 1600 rpm for 1 minute.

18. Seal the TCP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and transfer the plate to the post-PCR C1000 thermal cycler. Incubate the TCP plate at 95°C for 1 minute, followed by 35 cycles of 95°C for 0.5 minutes and 72C for 3.5 minutes, followed by 72°C for 5 minutes, followed by 10C forever, using the C1000 Thermal Cycler program 1_TCP_35 to amplify the ligated DANSR assays using universal PCR primers. The TCP can remain at 10°C on the thermal cycler overnight or can be stored at -20°C (-30 to -15ºC) for up to 3 days.

Microarray-Based Quantification of DANSR Assay UPCR Products

1. Refer to the Ariosa cell-free DNA System User Guide (1200000414595) for detailed instructions on executing the DANSR assay detection process using the Detection Robot.

2. Obtain the TCP plate from the C1000 thermal cycler or from -20°C storage, and allow the plate to equilibrate to room temperature. Centrifuge the TCP at 1000 rcf for one minute. Gently remove the seal from the plate in one steady, continuous motion to minimize splashing or carryover between wells. Avoid shaking or lifting the plate.

3. Execute the Director “Inoc 2 TCP” task on the Detection Robot to add Universal PCR Mix 2 (UPM2) to the TCP. In the “Inoc2 TCP” task, the robot transfers 48µL of UPM2 to each well of the TCP plate and performs 2 pipette mixes.

4. Seal the TCP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the TCP plate at 95°C for 1.5 minutes, followed by 72°C for 8.5 minutes, followed by 10°C forever, using the C1000 Thermal Cycler program 2_TCP_01 to further amplify DANSR assay UPCR products. Proceed directly to Make PPP task.

5. Obtain the TCP plate from the thermal cycler, and allow the plate to reach room temperature. Centrifuge the TCP at 1000 rcf for one minute. Gently remove the seal from the plate in one steady, continuous motion to minimize splashing or carryover between wells n. Avoid shaking or lifting the plate.

6. Execute the Director “Make PPP” task on the Detection Robot to purify and concentrate the DANSR assay UPCR products in the TCP plate, to transfer the concentrated UPCR product to a new plate containing PPP reagent, and to digest the DANSR assay UPCR products. In the PPP task, the robot transfers 70µL of each DANSR assay UPCR product in the TCP plate to the corresponding well of an AM3 plate containing 144µL of a paramagnetic sphere-containing solution (AM3). After incubating the AM3 plate for 5 minutes with mixing at 2000rpm and 5 minutes without mixing, the robot incubates the AM3 plate for 5 minutes on a neodymium magnet to immobilize the AM3 beads near the bottom of the wells. After removing the supernatant from each well of the AM3 plate, the robot washes the AM3 beads with 150µL 70% EtOH (ETH), and then elutes the DANSR assay UPCR products from the beads by dispensing 24µL Resuspension 7 Buffer2 (R7B2) to each well. After incubating the AM3 plate for 1 minute with mixing at 1200rpm, the robot incubates the AM3 plate on a magnet for 5 minutes to immobilize the AM3 beads near the bottom of the wells, and transfers the DANSR assay UPCR products-containing R7B2 supernatant to a new PPP plate.

7. Seal the PPP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the PPP plate at 37°C for 6 hours, followed by 95°C for 3 minutes, followed by 4°C forever, using the C1000 Thermal Cycler program Inc_PPP to digest the DANSR assay UPCR products. The PPP can remain at 4°C on the thermal cycler overnight or can be stored at -20°C (-30 to -15ºC) for up to 3 days.

8. Obtain the PPP plate from the thermal cycler or from -20°C storage, and allow the plate to reach room temperature. Centrifuge the PPP at 1000 rcf for one minute. Gently remove the seal from the plate in one steady, continuous motion. Avoid shaking or lifting the plate.

9. Execute the Director “Consolidate 96-AOP” task on the Detection Robot to hybridize the digested DANSR assay UPCR products to a new Array of Pegs (AOP). In the Consolidate 96-AOP task, the robot transfers 11µL of Hyb Anneal Buffer (HAB) to each well of the PPP and shakes the PPP at 2100 rpm for 1 minute. The robot then transfers 30µL of liquid from each well of the PPP to the corresponding well of a new Stain Tray (ST). The robot then couples a new AOP to the ST, creating a AOP-ST hybridization assembly.

10. Remove the AOP-ST hybridization assembly from the Detection Robot and use 4 array clips to secure the array to the stain tray. Incubate the AOP-ST hybridization assembly at 700 rpm at 70°C for 30 minutes, followed by incubation at a gradually decreasing temperature from 70°C to 35°C over the course of 2 hours in a Binder KB53 oven. After incubation is complete, retrieve the AOP-ST assembly from the Binder KB53 oven, and remove the array clips. Carefully break the seal between the AOP and the ST using a screwdriver.

11. Execute the Director “Label AOP” task on the Detection Robot to label the array using a fluorescently labeled oligonucleotide probe. In the “Label AOP” task, the robot washes the AOP in 35mL Post-Hyb Wash Buffer (PHB). The robot then transfers the AOP to a reagent tray containing 22mL Array Label Mix (ALM), and incubates the ALM tray-AOP assembly at 37°C for 2 hours in a Tecan Monitored Incubation Option (MIO) incubator. The robot then dispenses 175µL Array Imaging Buffer (AIB) into each well of a Plate Scan Tray (PST), washes the AOP in 35mL Post Label Buffer (PLB), transfers the AOP to the PST, and shakes the AOP-PST assembly at 500 rpm for 5 minutes.

12. Execute the Director “Load AIS” task on the Concerto Imager to log the imaging of the AOP. Enter the barcodes of the AIS (Array Imaging System, the Concerto Imager), the AOP, and the PST into Director software, and select Save.

13. Use the Concerto Imaging System software to image the AOP. Refer to the AcfS User Guide for instructions on operating the Concerto Imager. . Launch the Concerto Imaging System software from the Windows Start Menu. Once the application has launched, press Start to open the Imager drawer. Taking care not to spill AIB, place the AOP-PST assembly into the Imager drawer with the barcode facing to the rear of the instrument. Verify the AOP-PST assembly is seated securely on the drawer tabs and select Load in the Imager application to retract the drawer. The Imager scans the AOP barcode. Select Start in the Imager application to initiate the imaging process.

14. Imaging takes about 1 minute per array. Imaging starts with the top left array. All arrays on the AOP are imaged. Four images are taken of each array, and the Concerto Imaging System software combines the four images from each array into a single DAT file. The software thus generates 96 DAT files per AOP, one DAT file per array. The software analyzes these 96 image files to create 96 corresponding CEL files containing intensity values for each feature. The software saves these 96 DAT and CEL files on the Concerto Imager computer. Once imaging is complete, select Unload in the Imager application to open the Imager drawer. After removing the AOP-PST assembly, select Load to retract the drawer. It is recommended to remove the AOP-PST assembly upon completion of scanning. Leaving a scanned plate inside the imager for a prolonged period may result in reagent precipitation potentially causing damage to the imager.

FORTE Algorithmic Analysis

1. Execute the Director "Finish AIS" task on the Concerto Imager workstation to initiate analysis by the FORTE algorithm. In the "Finish AIS" task, Director copies the 96 DAT and CEL files from the AOP to the Analysis Server. The Analysis Service then instructs the FORTE_R.DLL application to perform the analysis by the FORTE algorithm using the CEL files and the corresponding Specimen Sample Sheet.

2. The FORTE algorithm computes capped adjusted trisomy probability scores and probability classifications for T13, T18, and T21; capped sex chromosome aneuploidy probability scores and classification; 22q11.2 deletion classification; and fetal sex for each of the samples and APC in the run.

QC Review and Reporting of Results

1. Execute the Director Create Reports task to review run QC, sample QC, and probability results for the samples and APC. Select the run to review based upon its NAP barcode. Once a run is selected, the "Create Reports" task populates the interface with the corresponding results.

2. The FORTE algorithm computes a set of 4 run ("Lane") QC metrics for each run of 48-96 samples (i.e., a "lane" defined as a batch of 48-95 individual samples and one APC control), and 6 sample QC metrics for each sample. The FORTE algorithm determines QC pass/fail status for each run and sample using pre-established acceptance criteria. The QC metrics and their acceptance criteria are presented in Tables 13 and 14 below.

Table 13: Run QC Metrics

Run QC Metric	Description	Minimum Criteria	Maximum Criteria
LaneMedianSignal	Median signal across the array.	200	10000
LaneFracTestSamplesPassed	Fraction of test samples that passed sample QC metrics.	0.49	1.0
LaneSignalToNoise	A measure of signal and variance across the entire array.	0.9	1
LaneNoise	A measure of the amount of variance observed across the array.	0.9	1

Table 14: Sample QC Metrics

Sample QC Metric	Description	Minimum Criteria	Maximum Criteria
ArrayQuality	An indication of the overall quality of the array. Manufacturing defects on the array may occur at rate of 4% and cause this metric to fail if a large portion of the array is affected by the defect.	0.9	1
Signal	The signal in each sample.	200	10000
FetalFraction	An estimation of the fetal fraction.	0.04	0.6
SampleIntegrity	An indication of the overall quality of the sample.	0.9	1
Noise	A measure of the variance observed within the sample.	0.9	1
SignalToNoise	A measure of the relationship between the signal and the variance within the sample.	0.9	1

3. If any lane QC metric fails, the “ReportRedraw” check box is selected for all of the samples within the lane. This queues the samples for a redraw report to be created. If a sample passes all lane QC metrics and fails any sample QC metric, the “ReportRedraw” check box is selected for the sample. This queues the sample for a redraw report to be created. If a sample passes all lane QC metrics and passes all sample quality metrics, the report type “ReportData” check box is selected. This queues the sample for a standard report to be created.

4. The second tube should only be run when the first tube does not yield a test result for the probability of trisomy 21, 18 and 13. However, if the first tube fails the QC metric for FetalFraction, request a redraw rather than run the second tube (as the likelihood of failing the Fetal Fraction QC metric again is high).[6]

5. Do not run the second tube when the first tube does not yield a test result for the probability of sex chromosome aneuploidy or fetal sex (but does yield a test result for the probability of trisomy 21, 18 and 13).

6. The DANSR Kit US-RUO provides an APC to be included in each run. The APC is derived from genomic DNA isolated from buffy coats of one female and one male human subject (see demographic data in Table 17). The expected values for the APC are presented in Table 15 (male sex and low probability for trisomy). If the APC passes sample QC and does not yield expected results, select ReportRedraw for all samples. Otherwise, proceed to report results for the 48-95 specimens. See Table 15 below for further clarification on use of the APC:

Table 15: Use of the Control

	Sample QC Metric	**Aneuploidy Result**	**Outcome**
If APC	**Passes** all 6 sample QC metrics and…	is **low** probability for trisomy,	then, Report on samples within the batch
	Passes all 6 sample QC metrics and…	is **high** probability for one or more trisomy,	then, **do not** report on samples in the batch
	Fails one or more of the 6 sample QC metrics and…	is no result	Contact Roche Support

Table 16: Expected APC Results

Control DNA	**T13**	**T18**	**T21**	**Sex**
APC	Low	Low	Low	Male

Table 17: APC Demographic Data

Control DNA	Maternal Age (Years)	Gestational Age (Weeks)	Gestational Age (Days)	IVF Status	Egg Donor Age (Years)	Number of Fetus	Sample Type
APC	30	15	0	Non-Self	30	1	Control

Note: Putting in the incorrect number of fetuses or the incorrect IVF status for the APC will increase the likelihood of QC failure of the APC.

When the individual sample QC metrics and batch (lane) QC metrics are successfully met, they provide necessary and sufficient evidence that the test is performing appropriately.

PROCEDURAL PRECAUTIONS

1. As with any test procedure, good laboratory technique is essential to the proper performance of this test.

2. Due to the high analytical sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

3. Reliable results are dependent on appropriate specimen collection, transport, storage, and processing. Follow the procedures in this package insert.

4. Good laboratory practices and careful adherence to the procedures specified in this package insert are necessary to avoid contamination of reagents.

5. Due to inherent differences between technologies, it is recommended that prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences.

6. It is recommended that reagents from the same kit be used together.

7. The test requires use of 48-96 unique biological samples (including controls) per batch. Therefore, using fewer than 48 unique samples(not including controls) could affect test performance.

   NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples.

8. Processing more than one tube from the same biological sample in the same batch may negatively affect test performance.

9. Reassessing an entire lane is not recommended and may negatively affect test performance. It is only recommended to reassess specific samples that require correction of demographic information, or the addition/subtraction of optional tests such as SCAP.

10. Ensure demographic information is input correctly in order to generate accurate results. Changing this information for a second run could lead to discrepant results.

11. It is possible to obtain valid trisomy results with inconclusive SCAP results and/or Fetal Sex results. In such cases, testing should not be repeated.

LIMITATIONS

1. The DANSR Kit US-RUO is for Research Use Only. Not to be used in diagnostic procedures.

2. The test is not intended to be used in analysis of cfDNA from pregnancies with more than two fetuses, induced or spontaneous twin demise, mosaicism, partial chromosome aneuploidy, translocations, maternal aneuploidy, maternal transplant, or maternal malignancy.[7]

3. The test is compatible for use in analysis of cfDNA samples isolated from plasma from pregnant women of ≥ 18 years of age, of ≥ 10 weeks' gestation, and with ≤ 2 fetuses.

4. The test has been validated for use on specimens collected using the Roche Cell-Free DNA Collection Tube (PN 07785674001 or equivalent).

5. Use of this product must be limited to personnel trained in the techniques described in this package insert. Training is provided by Roche authorized personnel.

6. The test is validated for use with cfDNA isolated from > 2mL of plasma per specimen. Use of cfDNA isolated from 4mL of plasma per specimen is recommended.

7. The test requires at least 4% fetal cfDNA in order to provide a result. The cfDNA specimens containing excessive amounts of maternal cfDNA may affect the performance of the test.

8. Certain factors associated with samples, such as mode of conception (i.e., in vitro fertilization), lower gestational age, higher maternal weight and twin pregnancy may be associated with lower fetal fraction and, as a result, may have higher no-call rates. [8,9,10]

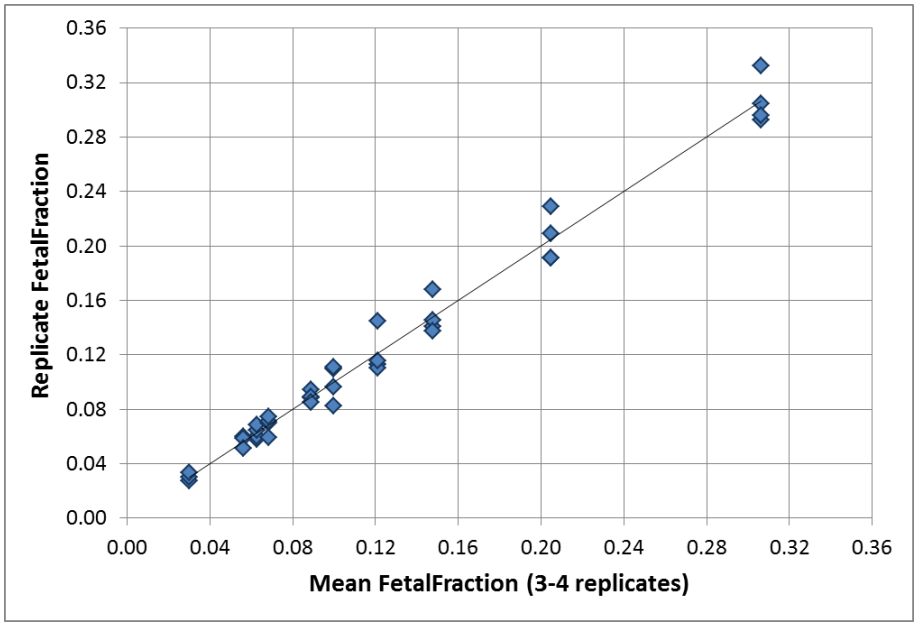
9. Sex chromosome aneuploidies have only been validated in using samples derived from singleton pregnancies.

10. 22q11.2 deletion has only been validated using samples derived from singleton pregnancies.

Fetal Fraction[6]

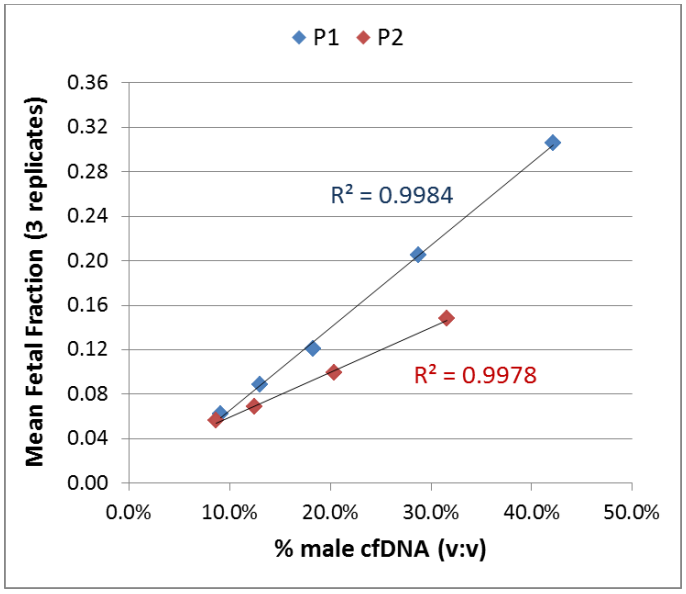
The test estimates the fraction of the cfDNA sample that originated from the fetus and reports the estimate as the Sample QC Metric FetalFraction. The test requires samples to have fetal fraction values of 4% or greater in order to provide a result. The analytical performance of the test fetal fraction metric was assessed by using the test to evaluate multiple replicates of a set of contrived pregnancy specimens, wherein plasma or cfDNA from plasma from related individuals, one male (contrived fetus) and one female (contrived mother), was mixed in specified proportions. Fetal fraction metrics were evaluated in the resulting data to characterize repeatability, linearity, and limit of detection. Repeatability was assessed by evaluating mixtures with ≥3 replicates at fetal fractions of 3% - 27% and calculating the coefficient of variation (CV) of the test fetal fraction metric at each of the tested fetal fractions. At all tested fetal fractions, the CV was < 10% (replicate data in Figure 1).

Figure 1: Repeatability of Fetal Fraction



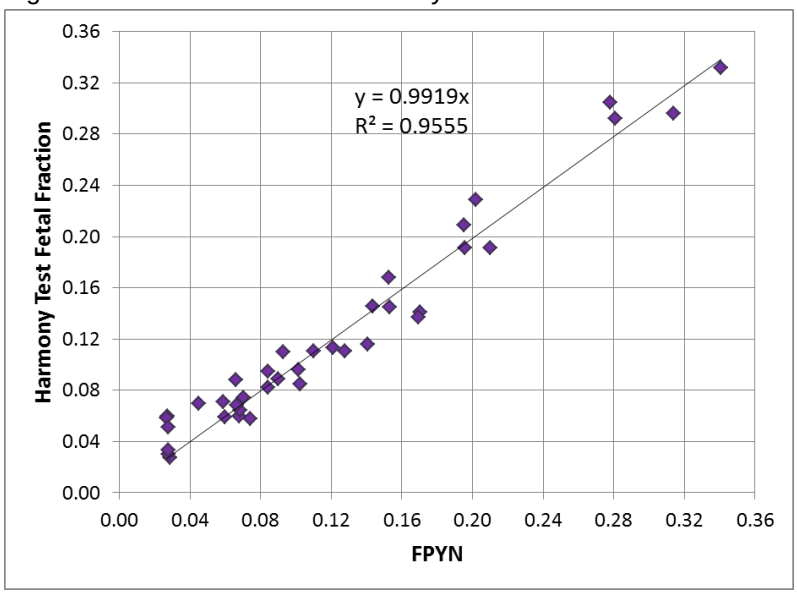
Linearity was evaluated by comparing the test fetal fraction metric obtained from each mixture with the percentage of total plasma volume in the mixture derived from the male individual. The correlation coefficient (R^2) of the linear regression of the FetalFraction/% male volume comparison was determined for two separate sets of contrived specimens with different subject sources (P1 and P2) with male:female mixtures titrated from 10% male to 45% male. Figure 2 illustrates the strong linear relationship for the two sets of mixtures (P1 and P2) for FetalFraction/% male volume ($R^2 > 0.99$).

Figure 2: Linearity of Fetal Fraction to % Male Volume in Male:Female Mixtures



The ability of the test to detect minor source cfDNA at fractions from 4% was illustrated in the same set of male:female mixtures by comparing the test Fetal Fraction metric to FPYN, an independent measure of fetal fraction which uses chromosome Y – specific loci (Figure 3).

Figure 3: Detection of Male cfDNA by the test



Plasma Input[8]

Over 28,000 blood specimens were collected into the Roche cfDNA tube or equivalent and were processed through the test within 7 days of the blood draw. The test specimens pass rate and distribution of fetal fraction were evaluated by input plasma volume for volumes from 2mL to 5mL to verify the effectiveness of the test for this range of specimen input volumes (distribution of specimen input volume is in Table 21).

Table 21 Specimen Input Volume

Plasma input (mL)	# samples
2.0-2.5	43
2.5-3.0	137
3.0-3.5	561
3.5-4.0	1932
4.0-4.5	6179
4.5-5.0	20077
Total	**28929**

The Effective Pass Rate for a particular plasma input volume was calculated as the ratio of the pass rate for the mL input volume divided by the pass rate of the total dataset. In addition, for the population of specimens at each plasma input volume, the mean and standard deviation of the test fetal fraction metric was calculated. As illustrated in Figure 4, the Effective Pass Rate was > 90% for all plasma input volumes from 2mL through 5mL; however, there is a clear inverse relationship of plasma input to test pass rate. In contrast, the plasma input volume had no effect on the fetal fraction distribution (Figure 5).

Figure 4: Effective Pass Rate by mL of Plasma Input

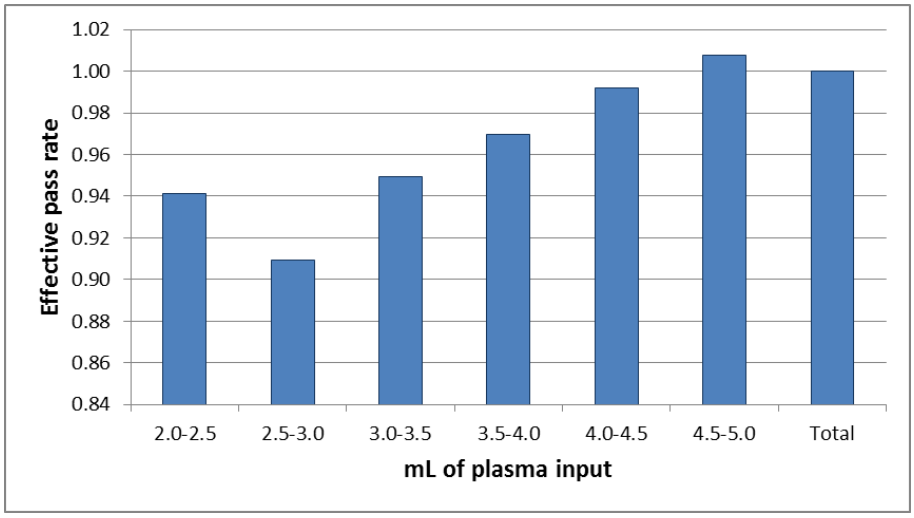
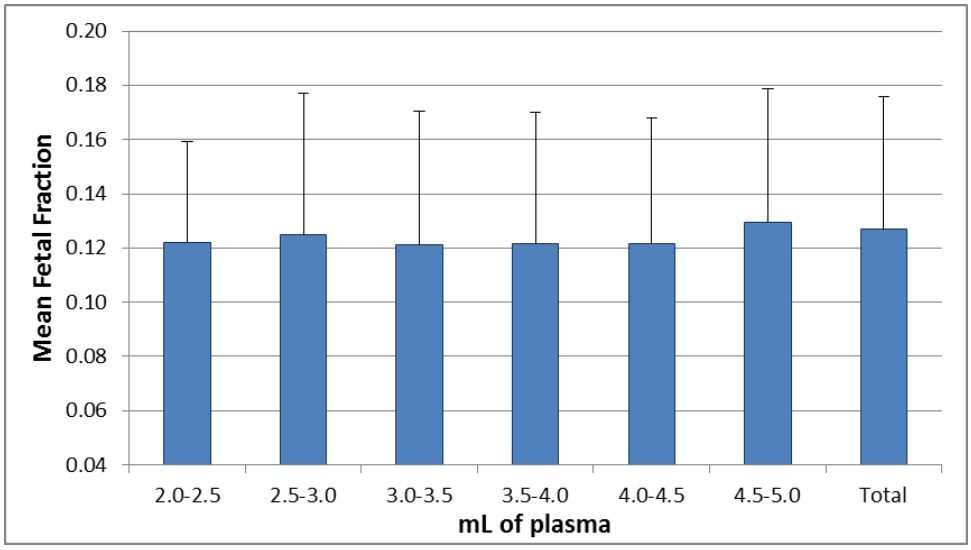


Figure 5: Fetal Fraction Mean and Standard Deviation by Plasma Input Volume



Standard deviation for each series is shown as error bars.

Interfering Substances[8]

To determine if genomic DNA released into the sample from white blood cell lysis could result in fetal cell free DNA fraction failure, replicate samples from 170 subjects were tested. Samples for each individual subject were collected at the same time and included whole blood collected in the Roche cell-free DNA collection tube.

The results from the study showed that the extent of the difference in hemoglobin (Hb) level between samples from the same blood draw do not correlate with any changes in assay pass rate or fetal fraction, even if Hb levels were above 500 mg/dL.

No significant differences were observed in fetal fraction across a range of Hb levels so it is not necessary to reject specimen plasma samples due to hemolysis since plasma discoloration caused by hemolysis is not correlated with white blood cell lysis.

The difference in fetal fractions between each sample pair tested was also determined and the results demonstrated no significant difference in fetal fraction in samples across a range of Hb levels.

PERFORMANCE CHARACTERISTICS

Associated Performance Metric

The six QC metric used to pass or fail a sample are each comprised of several component metrics also called as performance metrics that are incorporated into a single value. In total, fifteen independent measures of quality (performance metrics) are evaluated for each sample. Evaluation of these performance metrics can provide insight to support troubleshooting runs or longitudinal monitoring. These performance metrics are not intended to be used as QC metrics.

Metric Descriptions

Each sample QC metric has associated performance metrics listed. The table below (Table 22) lists the Sample QC metric along with their associated performance metric and the lane QC metrics.

Table 22. Associated Performance metrics

Metric Description	QC Metrics	Associated Performance Metric
Sample Metric	Array Quality	MinStarRobustSigSep
		MaxFracSaturated
		FracGoodFeatures
	Signal	MinNonPolyMedian Signal
	Sample Integrity	FracHom
		FracInfo
		CXXObservedMedianRatio
	FetalFractionQC	FetalFractionQC
	Noise	FPPolyRSE
		MaxNormLogRatioBias
		QuantNormAdjIQR
		AssayAngle
	SignalToNoise	MinNonPolyRobustSigSep
		MinSNR
		MinConfidence

QC metrics are presented in the order in which they should be interpreted; that is, if a sample fails a metric presented earlier in the list, the values of all later metrics are suspect and should not be interpreted to indicate what they are designed to capture. For example, if a sample fails SampleIntegrity, then FetalFractionQC is suspect and should not be viewed as an accurate estimate of the fetal fraction.

ArrayQuality
ArrayQuality provides a single metric evaluating array characteristics that are integral to the quality of the array data, especially with regards to imaging issues.
Possible causes include:

- The wrong array design being used, physical damage to the array, or laboratory processing issues.
- Manufacturing defects on the array may occur at rate of 4% and cause this metric to fail if a large portion of the array is affected by the defect.
- Large amounts of saturating signal on the array (possibly due to contaminating dust), extremely high background noise, or excessive sample signal that interferes with the ability to discriminate different levels of signal between features on the array
- A large fraction of array features have low signal or there is a high degree of variance across replicate features.

This metric is comprised of three internal metric performance metrics that evaluate the following characteristics and possible causes:

1. MinStarRobustSigSep

   A measure of whether signal was detected over the features on the array that are unique to the DANSR custom array design. If the wrong array design is used, this metric will be impacted.

2. MaxFracSaturated

   A measure of the fraction of the array that is overexposed or has saturating signal. If there are large amounts of saturating signal on the array due to contaminating particles on the array, extremely high

background noise, or excessive signal from the sample that interfere with the ability to discriminate different levels of signal between features on the array, this metric will be impacted.

3. FracGoodFeatures

   A measure of the fraction of array features per sample that have non-saturating signal and low variance across replicate array features. If a large fraction of array features have low signal, high variance across replicate features, or no signal at all, this metric will be impacted.

Signal
Signal measures the strength of the informative fluorescence on the array. Possible causes include:

- Insufficient cfDNA in the sample.
- Loss of DNA during processing.
- Loss of signal due to insufficient labeling of the sample.
- Insufficient hybridization of the sample to the array.
- Underexposure of the image. Failures can be caused by:
  - Poor cfDNA extraction.
  - Insufficient reagents at certain points of the process.
  - Expired reagents.
  - An expired imaging lamp.

SampleIntegrity
SampleIntegrity provides a single metric evaluating the sample's suitability for analysis based on intrinsic properties of the sample's genetic material, cell-free DNA (cfDNA).
Possible causes include:

- Cross-contamination with another sample.
- The sample has incorrect sample demographic information (e.g., twins instead of a singleton, or nonself egg donor).
- Extremely low or no fetal fraction (for example, a nonpregnant sample).

This metric is comprised of three internal performance metrics that evaluate the following characteristics and possible causes:

1. FracHom

   A measure of the polymorphic assays based on the genetic knowledge of the relationship of the mother and fetus and in the number of fetuses. Samples which are cross contaminated with another sample, or if the sample has an incorrect demographic (e.g. twins instead of single fetus), or where the genotyping is compromised due to large amounts of array noise will impact this metric. Furthermore, samples which are incompatible with the validated test due to greater than 2 fetuses will impact this metric.
2. FracInfo

   A measure of the polymorphic assays that are used to estimate fetal cfDNA. Samples with extremely low or no fetal fraction will impact this metric, or where the genotyping is compromised due to insufficient resolution of signal in the array.
3. CXXObservedMedianRatio

A measure of the amount of signal from the X chromosome from a sample. If the major cfDNA source of the sample is male then this metric will be impacted.**Noise**
Noise provides a single metric comprised of four orthogonal internal performance metrics that evaluate the following noise characteristics and possible causes.

Possible modes of failure include:

- Failures in the reagents or processes during the library preparation process (for example, poor biotinylation of the cell-free DNA, poor annealing of the DANSR assays to the sample, or nonspecific ligation during Ligate UNA)

- Errors in laboratory processes that might result in yield loss.

This metric is comprised of four internal performance metrics that evaluate the following characteristics and possible causes:

1. FPPolyRSE

   A measure of the variance in the fetal fraction estimate. This metric is impacted when there is not enough sample diversity in the batch.
2. MaxNormLogRatioBias

   A measure of the variance within a sample for signals from nonpolymorphic assays. This metric will be impacted if there is insufficient initial cfDNA from the specimen or processing issues during library preparation, especially during hybridization of the sample to the DANSR assays.
3. QuantNormAdjIQR

   A measure of the amount of difference of an individual sample's data from the array data of the batch of samples (lane) in which the sample was processed. This metric will be impacted if a sample is substantially unlike the other samples on the array. This may occur due to errors in laboratory processing that only affect individual wells, such as a bubble in pipetting that leads to insufficient amount of a reagent dispensed.
4. AssayAngle

   A measure of the inconsistency of a sample's data from the model that a fetus may only have a single trisomic chromosome. This metric may be impacted if the sample has more than one aneuploidy, unusual genomic conditions, or the genetic material of the sample is substantially unlike the other samples on the array.

SignalToNoise

SignalToNoise provides a measure of the relationship between the signal and the variance within the sample. This metric is comprised of four three internal performance metrics that evaluate the following characteristics of signal to noise:

1. MinNonPolyRobustSigSep

   A measure of the difference between assay signal on the array from non-specific signal on the array. If the non-specific background signal on the array is too high or the signal from the assays is too low, this metric will be impacted. Issues in reagents or processes during the detection process, especially during hybridization of the sample to the array will impact this metric.
2. MinSNR

   A measure of the minimum signal to noise ratio observed over all the assays within a sample. This metric considers the allowable amount of variance across chromosomal assays based on the estimated fetal fraction. The allowable variance decreases as fetal fraction decreases. This metric will be impacted if the fetal fraction is too low or the variance across assays within a sample is too high. This metric is often impacted when several previous metrics pass but are near the thresholds.
3. MinConfidence

   A measure of the statistical certainty of the observed array data for a sample. If the data from a sample leads to results that are unreliable, this metric will be impacted. As All aspects of quality influence this metric.

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TABLE OF PART NUMBERS

Table 23. Roche P/Ns

Item Description	Roche P/N
DANSR US-RUO Kit	08318085001
Ariosa cell-free DNA System (AcfS) Software	08012300001
Library Robot	07759371001
Library Box 1	07759207001
Ariosa Magnetospheres 1 (AM1T)	07871473001
Ariosa Magnetospheres 2 (AM2T)	07871503001
Resuspend 2 Buffer (R2B)	07871538001
Ligate Equilibrate Buffer (LEB)	07871538001
Resuspend 4 Buffer 2 (R4B2)	07871546001
Ariosa Magnetospheres 3 (AM3T)	07871511001
Barcode (AD#-NAP) 8 ea	08798800001
Barcode (AD#-UNA) 8 ea	08798818001
Barcode (AD#-AM1) 8 ea	08798826001
Barcode (AD#-AM2) 8 ea	08798834001
Barcode (AD# -AM3) 8 ea	08798842001
Barcode (AD#-TCP) 8 ea	08798893001
Barcode (AD#-PPP) 8 ea	08798907001
Barcode (AD#-ETH) 2 ea	08798958001
Detection Box 1	07759215001
Resuspend 7 Buffer 2 (R7B2)	08482764001
Array Imaging Buffer (AIB)	08482772001
Post Hyb Wash Buffer (PHB)	08482799001
Post Label Wash buffer (PLB)	08484392001
Array of Pegs (AOP 96)	07955618001
Hyb Anneal Buffer Trough Barcode	08799032001
Array Imaging Buffer Trough Barcode	08799105001
Concerto Imager 120V	08088900001 or 09337407001

Item Description	Roche P/N
Ariosa Array 96S384 Box 2	07871643001
Library Box 2	07759240001
Biotinylate Master Mix (BTM)	08482845001
Anneal DNA Buffer 3 (ADB3)	08482861001
Ligate Bead Buffer (LBB)	08482870001
Ligate Master Mix (LIM)	08482888001
Thermal Cycle Plate (TCPT)	08484015001
Purify and Prepare Plate (PPPT)	08484023001
Ligate Wash Buffer (LWB)	08482896001
Assay Performance Control (APC)	08798788001
Detection Box 3	07759231001
Stain Tray	08805024001
Scan Tray	08805032001
Blue Tray	07871759001
Gasket	07871767001
Trough Barcode (TD#-PHB) 4 ea	08798923001
Trough Barcode (TD#-PLB) 4 ea	08798931001
Trough Barcode (TD#-ALM) 10 ea	08798940001
Barcode (AD#-ETH) 2 ea	08798958001
Detection Box 4	07759258001
Universal PCR Mix 2 (UPM2)	08481849001
Array Label Mix (ALM)	08481857001
Hyb Anneal Buffer (HAB)	08481865001
Detection Robot	07759363001
Resuspend 7 Buffer 2 Trough Barcode	08798915001
Ethanol Trough Barcode	08798966001

GLOSSARY OF HARMONIZED SYMBOLS

Temperature limit

Batch code (Lot)

Use-by-date

Item number

Do not reuse

Manufacturer

Contains Sufficient for N tests

Consult Instructions For Use

Date of Manufacture

Do not dispose of electronic products in the general waste stream (for Concerto Imager only)

GHS08 Health Hazard: H360 May damage fertility or the unborn child

ACMA standards compliance label (for Concerto Imager only)

Global Trade Item Number

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Santa Clara, CA 95050 USA

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TECHNICAL SUPPORT

Please contact your local Roche Representative.