Harmony® IVD Kit

P/N 08011281001

AMB Protocol Supplemental Instructions for Use

FOR IN VITRO DIAGNOSTIC USE

CE
2797

Concerto Imager IVD

P/N 09101721001

Included with sales part numbers listed below:

P/N 09337423001 (230V) and P/N 09337393001 (120V)

CE IVD

PROFESSIONAL USE

Roche Sequencing Solutions, Inc
2821 Scott Blvd
Santa Clara, CA 95050 USA

Contents

BACKGROUND

This AMB protocol is intended to be used only by UK and Germany customers. The AMB protocol uses the AMB reagent in place of the the AM1 and AM2 reagents used in Library Box 1. For the AMB protocol, Library Box 1 will be replaced by Library Box 1B. The other reagents and boxes will remain unchanged from the original configuration. This form contains specific procedures for AMB protocol. All other sections/ procedures should refer back to 10100648001.

The AMB protocol has not been validated for the 22q deletion test option. Customers using the AMB protocol should not report results for the 22q test.

MATERIALS PROVIDED

Table 1 lists the components of the DANSR Library Box 1B.

Table 1. DANSR LIBRARY BOX 1B (P/N 09212159001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
AMBT	Ariosa Magnetospheres B	250 mL Bottle	150 mL	2	2ºC to 8ºC Library Lab	09212132001
AM3T	Ariosa Magnetospheres 3	250 mL Bottle	200 mL	1	2 ºC to 8ºC Library Lab	08482756001
R2B	Resuspend 2 Buffer	250 mL Bottle	175 mL	1	2ºC to 8ºC Library Lab	08482691001
LEB	Ligate Equilibrate Buffer	500 mL Bottle	300 mL	1	2ºC to 8ºC Library Lab	08482721001
R4B2	Resuspend 4 Buffer 2	250 mL Bottle	200 mL	1	2ºC to 8ºC Library Lab	08482748001
#NAP	Nucleic Acid Plate Barcode	Barcode	N/A	8	20ºC to 25ºC Library Lab	08798800001
#UNA	96-well UNA Plate Barcode	Barcode	N/A	8	20ºC to 25ºC Library Lab	08798818001
#AMB	AMB Plate Barcode	Barcode	N/A	16	20 to 25°C Library Lab	09212116001
#AM3	AM3 Plate Barcode	Barcode	N/A	8	20ºC to 25ºC Library Lab	08798842001
#TCP	Thermal Cycle Plate Barcode	Barcode	N/A	8	20ºC to 25ºC Library Lab	08798893001
#PPP	Purify & Prepare Plate Barcode	Barcode	N/A	8	20ºC to 25ºC Library Lab	08798907001
#ETH	Ethanol Barcode	Barcode	N/A	2	20ºC to 25ºC Library Lab	08798958001
#TD-AMBT	Ariosa Magnetospheres B Trough Barcode	Barcode	N/A	32	20 to 25°C Library Lab	09212124001
#TD-AM3T	Ariosa Magnetospheres 3 Trough Barcode	Barcode	N/A	16	20ºC to 25ºC Library Lab	08798885001
#TD-ETH	Ethanol Trough Barcode	Barcode	N/A	16	20ºC to 25ºC Library Lab	08798966001
#TD-R2B	Resuspend 2 Buffer Trough Barcode	Barcode	N/A	16	20ºC to 25ºC Library Lab	08798974001
#TD-LEB	Ligate Equilibrate Buffer Trough Barcode	Barcode	N/A	16	20ºC to 25ºC Library Lab	08798982001
#TD-LWB	Ligate Wash Buffer Trough Barcode	Barcode	N/A	16	20ºC to 25ºC Library Lab	08799008001
#TD-R4B2	Resuspend 4 Buffer 2 Trough Barcode	Barcode	N/A	16	20ºC to 25ºC Library Lab	08799016001

# indicates barcode labels

Table 2. Active Ingredients in Library Box 1B and Safety Information

Kit	Active Ingredients	Safety Symbol and Warning
DANSR LIBRARY BOX 1B	Ariosa Magnetospheres (AMBT) 100% Magentic bead reagent	N/A
	Ariosa Magnetospheres 3 (AM3T) 100% Ariosa Magnetospheres 3	N/A
	Resuspend 2 Buffer (R2B) Buffered aqueous solution	N/A
	Ligate Equilibrate Buffer (LEB) Buffered aqueous solution	N/A
	Resuspend 4 Buffer 2 (R4B2) < 1% Primers in buffered aqueous solution	N/A

PRECAUTIONS, HANDLING REQUIREMENTS AND LIMITATIONS

Refer back to 10100648001 for details on precautions, handling requirements, and limitations.

Safety information

Safety Data Sheets (SDSs) for the Harmony IVD Kit can be obtained from https://pim-eservices.roche.com/eLD/web/us/en/home

Table 3: Part Number for SDS

Kit Component	Roche P/N
Harmony® IVD Kit DANSR™ Library Box 1B	09212159001

Handle and dispose of Harmony IVD Kit reagents according to the SDSs. Appropriate precautions (including use of personal protective equipment (PPE)) should be used when handling and disposing of Harmony IVD Kit 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 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 LIBRARY BOX 1B, verify the contents of the kit using the MATERIALS PROVIDED tables above.

IMPORTANT

If any Kit component is missing or damaged, contact your local Roche representative.

IMPORTANT

If the outer shipping container has been opened during shipping, contact your local Roche representative.

Store the DANSR LIBRARY BOX 1B components at temperatures indicated in the MATERIALS PROVIDED in to Table 1 above.

Expiration dates can be found on the reagents within the kit.

NOTE: The entire plate will be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary regardless of batch size.

AMBT and AM3T are provided in bulk containers, and must be dispensed into eight 96 well plates each (termed AMB and AM3 plates, respectively) prior to use, according to the Reagent Preparation Section of the IFU (see Figure 1 and Table 6). Dispensed AMB and AM3 plates have the same expiration date of their respective bulk container configurations when stored at 4°C (2 to 8°C). If stored at recommended temperatures and 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.

Table 4. Reagents that need to be dispensed

Component	Name	Supplied Reagent volume	Dispensed Quantity	Storage	Expiration	Roche P/N
AMBT	Ariosa Magnetospheres B	150 mL (x2)	280 ul dispensed 8 of 2.2mL 96 deep well plates	2°C to 8°C	Same as bulk reagent	09212132001
AM3T	Ariosa Magnetospheres 3	200 mL	144 ul dispensed 8 of 2.2mL 96 deep well plates	2°C to 8°C	Same as bulk reagent	08482756001

R2B, LEB, and R4B2 (see Table 5) 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.

Table 5. Reagents that are supplied in bulk container

Component	Name	Vessel	Volume	Quantity	Storage	Expiration	Roche P/N
R2B	Resuspend 2 Buffer	250 mL Bottle	175 ml	1	2°C to 8°C	Stated on the container	08482691001
LEB	Ligate Equilibrate Buffer	500 mL Bottle	300 ml	1	2°C to 8°C	Stated on the container	08482721001
R4B2	Resuspend 4 Buffer 2	250 mL Bottle	200 ml	1	2°C to 8°C	Stated on the container	08482748001

INSTRUCTIONS FOR USE

Professional Use Only

These instructions are for personnel who have received training from a qualified Roche representative.

Refer to the *AcfS User Guide* for details regarding operation and maintenance of the AcfS.

Reagent Preparation

Purpose

The Harmony IVD Kit is configured to enable analysis of 8 sets of 48-96 samples, including 48-95 plasma-derived cfDNA samples from pregnant women and 1 APC.

Reagent preparation tasks accomplish dispensing of 4 reagents that are shipped in bulk form into eight 96 well plates formatted for use in Director Software tasks executed by the Library Robot and the Detection Robot.

- AMBT
- AM3T
- TCPT
- PPPT

Reagent preparation of APC accomplishes dispensing of 1 APC that is shipped in bulk form into eight individual 1.5 mL tubes for storage purposes.

NOTE: These tasks do not have to be performed on the same day. Always perform 6 system washes in between tasks.

Figure 1 shows the workflow for Reagent Prep tasks that are performed on the Library Robot. The APC is aliquoted manually.

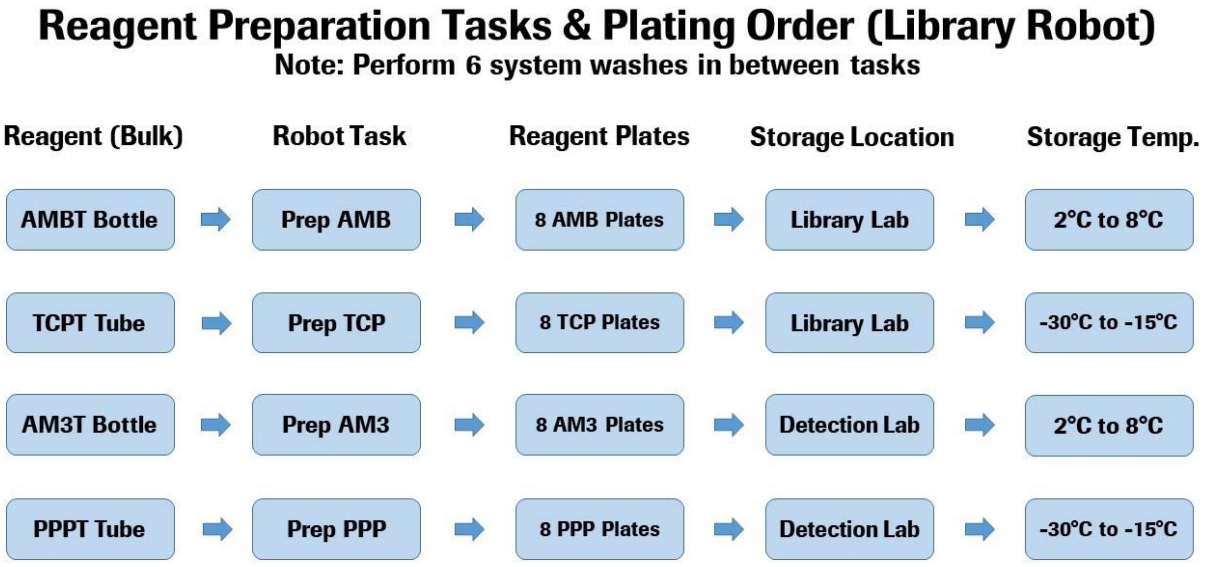

Figure 1. DANSR Assay Reagents Preparation Workflow

⚠ WARNING

Confirm reagents are not expired before use. Use of expired reagents can lead to a failed test.

IMPORTANT

Before beginning this task, equilibrate all reagents to room temperature (20°C to 25°C). This may take up to 2 hours. TCPT and PPPT may be removed from the -20°C freezer and stored at 2°C to 8°C overnight before room temperature equilibration. Do not allow reagents to remain at room temperature for more than 6 hours.

IMPORTANT

Failure to prepare reagents before beginning Reagent Tasks and/or failure to use the exact consumables listed in Table 6 will result in sample failure and damage to the robot.

Table 6. Required Materials for Executing Reagent Prep Tasks

Reagent	ID	Barcode Color	Vessel	Storage Temperature
Ariosa Magnetospheres B	AD-AMBT		250 mL bulk bottle (x2)	2°C to 8°C
Ariosa Magnetospheres 3	AD-AM3T		250 mL bulk bottle	
Thermal Cycle Plate	AD-TCPT		50 mL bulk tube	-15°C to -30°C
Purify and Prepare Plate	AD-PPPT		50 mL bulk tube	

Consumable	Number Used	Barcode Color
Barcode label for Ariosa Magnetospheres B trough (TD#-AMB)	1	
Barcode label for Ariosa Magnetospheres 3 trough (TD#-AM3)	1	
Barcode label for Ariosa Magnetospheres B (AD#-AMB)	8	
Barcode label for Ariosa Magnetospheres 3 (AD#-AM3)	8	
Barcode label for Thermal Cycle Plate (AD#-TCP)	8	
Barcode label for Purify and Prepare Plate (AD#-PPP)	8	
*Bio-Rad Adhesive plate seals	32	-
*PCR plate, hard shell, 0.2mL, 96-well	16	-
*Storage plate, square well, 2.2mL, 96-well	16	-
*Disposable trough, 320mL	2	-

**Required consumables but not provided with the kit*

Prep AMB

Purpose

In this task, eight AMB plates (2.2 mL, 96-deep-well, barcoded) with 280 µL of AMB in each well are generated from two bulk AMBT bottles for use in the Library Prep Step, Create UNA (See Create UNA). This reagent is used in the Library lab.

⚠ WARNING

PHYSICAL INJURY HAZARD. Before starting any robotic step, ensure that the front safety shield of the Library Robot is closed. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument.

IMPORTANT

Confirm that the AMBT reagent has equilibrated for two hours and is at room temperature (20°C to 25°C.) Reagent should be at room temperature to ensure proper robot dispensing.

Table 7. Required Consumables for preparing AMB Reagent

Consumable	Barcode Color	Number Used
Ariosa Magnetospheres B Reagent Barcode Label (AD#-AMB)		8
Ariosa Magnetospheres 1 Reagent Trough Barcode Label (TD#-AMB)		1
Plate, 96-deep, 2.2 mL, square well	NA	8
Disposable trough, 320 mL	NA	1
Adhesive plate seals	NA	8

Prepare AMB

Prepare the Library Robot for operation. Refer to the *AcfS User Guide*, “Before operating the AcfS”, for more details on robot preparation and loading.

Retrieve reagents and consumables needed for Prep AMB task. Refer to Table 6 and Table 8.

Table 8. Required Consumables for preparing AMB Reagent

Consumable	Barcode Color	Number Used
Ariosa Magnetospheres B Reagent Barcode Label (AD#-AMB)		8
Ariosa Magnetospheres 1 Reagent Trough Barcode Label (TD#-AMB)		1
Plate, 96-deep, 2.2 mL, square well	NA	8
Disposable trough, 320 mL	NA	1
Adhesive plate seals	NA	8

Confirm that the AMBT has been at room temperature for at least two hours. Visually inspect the vessel to see if it is the expected fill volume on the label.

Under Reagent Tasks, select **Prep AMB.**

Label eight new 2.2mL 96-deep well plates with the provided AMB barcode labels (AD#-AMB) on the right side of the plate, opposite of the lettered column. The barcode should be centered in the middle and flush with the top of the skirt (see Figure 2 for example).

Figure 2. Example of Barcode Label on Plate (AMB shown)

Figure 3. Example Barcode Label on Trough (AMB shown)

Label a 320 mL disposable trough with the provided AMBT barcode label (TD#-AMBT) on the right side of the trough. The barcode should be 3.5 cm from the top lip and 3 cm from the right vertical edge (see Figure 3 for example).

Place the trough in the 320 mL Tecan trough carrier position 1 with the barcode facing to the right and unobstructed (see Figure 4 for example).

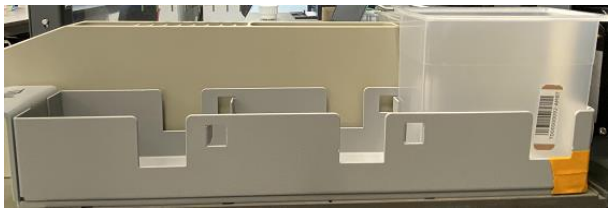

Figure 4. Trough in Carrier Example (AMB shown)

Arrange all equipment, consumables, and reagents on the trough, tube, and plate carriers as specified by worktable in the Prep AMB worktable (See Figure 5).

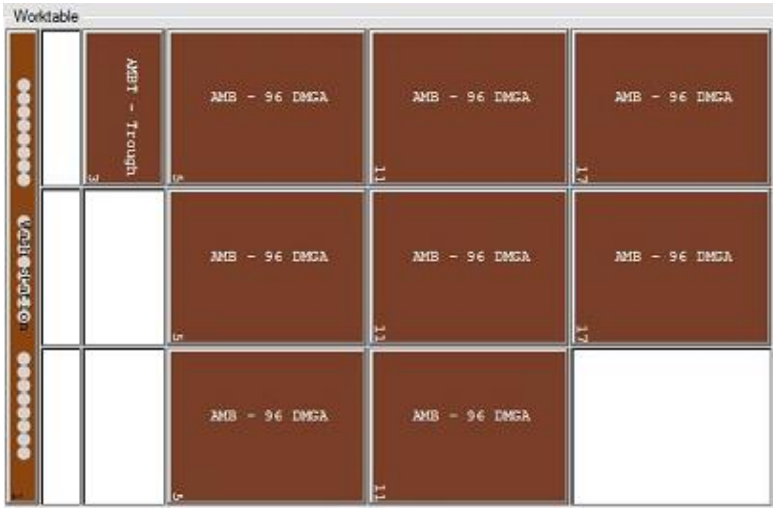

Figure 5. Prep AMB Worktable

Confirm that the reagent trough is barcoded and that the barcode is unobstructed and facing outwards towards the deck of the Library Robot.

Confirm that all reagent plates are oriented with well A1 in the upper-left corner of the plate carrier.

Confirm that the front safety shield to the Library Robot is closed. Perform three system washes.

After the system washes are complete, select **Run** to begin the task. Follow the prompts that appear in Director software.

Resuspend AMBT Beads

Invert the AMBT reagent bottle, whirl the AMBT reagent in a circular motion and then vigorously shake the bottle for at least one minute and until the beads are fully resuspended. Inspect the bottle to ensure that all beads are fully resuspended.

IMPORTANT

Ariosa Magnetospheres reagents MUST BE thoroughly resuspended to ensure that they are uniformly dispensed across all eight plates.

IMPORTANT

If not all beads are resuspended, repeat resuspension procedure until they are resuspended. If you are unable to fully resuspend beads, please contact your local affiliate.

IMPORTANT

Make sure to thoroughly adhere the seal to the plate. Ensure that all edges of the seal are in contact with the plates. Failure to do so can result in evaporation of the reagent leading to a failed test.

To continue, select **OK** in Director Software.

Scan and Pour AMB

When prompted, slide the 320 mL trough carrier out from the deck and scan the AMB trough barcode (TD#-AMBT).

Scan one AMBT reagent bottle (AD#-AMBT).

Scan the second AMBT bottle (AD#-AMBT).

Record the lot numbers of the AMBT reagent for reagent lot tracking and trending.

Pour the entire contents of the AMBT reagent bottles into the reagent trough.

Place the trough carrier back onto the deck.

IMPORTANT

Take care to not spill the reagent when transferring onto the robot deck.

Confirm that the front safety shield to the Library Robot is closed.

To continue, select **OK** in Director Software.

Store the AMB Plates

Upon completion of the robot action, remove the AMB plates from the Library Robot.

Use Speedball brayer to thoroughly adhere seal to AMB plates.

Visually check the AMB plates for liquid uniformity.

Remove the AMBT trough from the robot deck and visually check the residual volume. There should be at least 20 mL of reagent remaining in the trough.

If you notice an inconsistency with the vessel fill volume, refer to the AcfS User Guide, "Troubleshooting" for more information.

Transfer the AMB plates to 4°C (2°C to 8°C) storage for use in Director Library Tasks (see Library Generation Tasks).

AMB plates have the same expiration date as their bulk container configuration when stored at 4°C (2°C to 8°C).

IMPORTANT

Carefully handle the AMB plate when transferring the plate to storage. Avoid splashing the reagent onto the adhesive seal. Splashing reagent onto the adhesive seal can change the volumes, which can impact performance during the Create UNA Step. Visually inspect the seal for droplets. If splashing occurs do not use the plate.

IMPORTANT

Barcoded troughs can be washed and reused for up to a month.

Perform Cleanup

Refer to the *AcfS User Guide*, “After operating the AcfS” for details on how to prepare the Library Robot for the next task.

Thoroughly rinse the 320 mL trough with deionized water and placed in a clean area to dry. It is recommended to replace the trough monthly.

Prep AM3

Purpose

In this task, eight AM3 plates (2.2 mL, 96-deep-well, barcoded) are generated from a bulk AM3T bottle. Complete the Prep AM3 task in the Library Lab and then transfer the AM3 reagent plates to the Detection lab for storage.

- Before entering the Detection laboratory, ensure that you have generated enough plated reagents for use in the Detection laboratory.
- After you have entered the Detection laboratory, you cannot return to the Library laboratory.

WARNING

PHYSICAL INJURY HAZARD. Before starting any robotic step, ensure that the front safety shield of the Library Robot is closed. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument.

IMPORTANT

Confirm that the AM3T reagent has equilibrated for two hours and is at room temperature (20°C to 25°C) per step 3. Reagent should be at room temperature to ensure proper robot dispensing.

Prepare AM3

Prepare the Library Robot for operation. Refer to the *AcfS User Guide,* "Before operating the AcfS", for more details on robot preparation and loading.

Refer to Table 6 and Table 9 for reagents and consumables required for this step.

Table 9. Required consumables for preparing AM3 reagent

Consumable	Barcode Color	Number Used
Ariosa Magnetospheres 3 Reagent Barcode Label (AD#-AM3)		8
Ariosa Magnetospheres 3 Reagent Trough Barcode Label (TD#-AM3T)		1
Plate, 96-deep, 2.2 mL, square well	NA	8
Disposable trough, 320 mL	NA	1
Adhesive plate seals	NA	8

Confirm that the AM3T has been at room temperature for at least two hours. Visually inspect the vessel to see if it is the expected fill volume on the label.

Double-click the Director Software icon on the computer desktop.

In Director Software, select Reagent Tasks Prep AM3.

Repeat procedures in Prep AMB (Prep AMB) EXCEPT apply AD#-AM3 barcodes to eight plates and apply the TD#-AM3 barcode to a trough. Reference Figure 6.

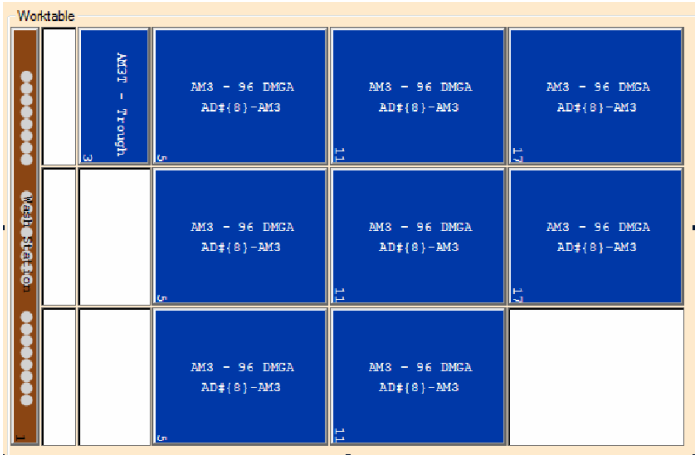

Figure 6. Prep AM3 Worktable

Resuspend AM3T Beads

Repeat resuspension procedure in Resuspend AMBT beads (see Resuspend AMB T Beads) EXCEPT invert the AM3T reagent bottle instead.

Scan and Pour AM3T

When prompted, slide the 320 mL trough carrier out from the deck and scan the AM3 trough barcode (TD#-AM3T).

Scan the AM3T reagent bottle (AD#-AM3T).

Record the lot number of the AM3T reagent for reagent lot tracking and trending.

Pour the entire contents of the.AM3T reagent bottle into the reagent trough.

Place the trough carrier back onto the deck.

IMPORTANT

Take care to not spill the reagent when transferring onto the robot deck. If spill occurs, see User Guide.

Confirm that the front safety shield to the Library Robot is closed.

To continue, select **OK** in Director Software.

Store the AM3 Plates

Upon completion of the robot action, remove the AM3 plates from the Library Robot.

Use Speedball brayer to thoroughly adhere seal to AM3 plates. *Ensure that all edges of the seal are in contact with the plate*.

Visually check the AM3 plates for liquid uniformity.

Remove the AM3 trough from the robot deck and visually check the residual volume. There should be at least 50 mL of reagent remaining in the trough.

If you notice an inconsistency with the vessel fill volume, refer to the AcfS User Guide, "Troubleshooting" for more information.

Transfer the AM3 plates to 4°C (2°C to 8°C) storage for use in Director Software Detection Tasks (see DANSR Assay Detection Workflow).

AM3 plates have the same expiration date as their bulk container configuration when stored at 4°C (2°C to 8°C).

IMPORTANT

Carefully handle the AM3 plate when transferring the plate to storage. Avoid splashing the reagent onto the adhesive seal. Splashing reagent onto the adhesive seal can change the volumes, which can impact performance of impact wells during the Make PPP Step. Visually inspect the seal for droplets. If splashing occurs do not use the plate.

Perform Cleanup

Refer to the AcfS User Guide, “After operating the AcfS” for details on how to prepare the Library Robot for the next task.

Thoroughly rinse the 320 mL trough with deionized water and placed in a clean area to dry. It is recommended to replace the trough monthly.

Refer back to 10100648001 for Prep TCP, Prep PPP, and Prep APC instructions.

Nucleic Acid Plate (NAP) prep

Purpose

In this step the Nucleic Acid Plate (NAP) is generated. A NAP is the plate containing the sample to be processed on the AcfS workflow and is the input for the Create UNA (Unified Nucleic Acid) task, a Library task in Director Software. Provide a NAP containing the 48 to 95 cfDNA samples and the Assay Performance Control (APC) at 150 µL in each well and at known locations within the NAP.

Refer back to 10100648001 for instructions on Nucleic Acid Plate (NAP) prep.

Library Generation Tasks

Purpose

These instructions are for only experienced personnel trained on the Library Robot, that has been installed and qualified by service personnel. These tasks are performed in the pre-PCR Library Lab (Figure 7).

Refer to the *AcfS User Guide* for an overview of: the AcfS system, AcfS Software, reagents, consumables and accessories, preparing for a workflow run, what to do before operating the AcfS, operating the AcfS, and what to do after operating the AcfS.

IMPORTANT

Confirm reagents are not expired before use. Use of expired reagents can lead to a failed test.

IMPORTANT

Prepare AMB and TCP reagents must be processed into reagent plates before starting the library workflow. The Director Software will not progress with out the required reagent plates. For instructions on preparing reagents for use in DANSR assay Library Tasks, see Reagent Preparation.

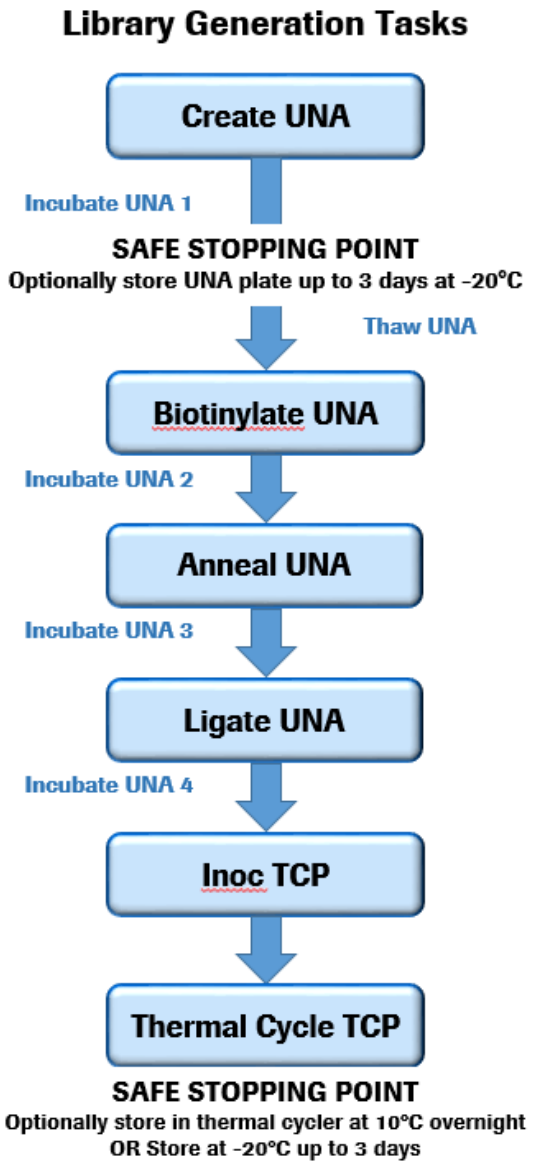

Figure 7. Library Generation Tasks overview

Create UNA

Purpose

In this task, 49-96 DNA samples in a NAP are concentrated and transferred to a UNA 96-well plate. The DNA is then rendered single-stranded in preparation for the library generation process. See Figure 7 for an overview of the steps.

Required Materials for Create UNA

Table 10. Required Reagents, Consumables, Equipment and Labware for Executing for Create UNA

Reagents / Consumables	Barcode Color	Vessel	Quantity Used	Storage
NAP (Accessioned plate containing patient samples) (AD#-NAP)		Plate	1	Not stored
AMB (AD#-AMB)		Plate	1	2°C to 8°C
R2B (AD#-R2B)		Bottle	15 mL	2°C to 8°C
ETH (AD#-ETH)		Container	35 mL	20°C to 25°C
ETH Barcode (TD#-ETH)		1	1	NA
R2D Barcode (TD#R2B)		NA	1	NA
UNA Barcode (AD#-UNA)		NA	1	NA
Adhesive plate seals	NA	NA	2	NA
Bio-Rad 96-well, hard shell PCR plate	NA	Plate	1	NA

Equipment	Quantity used	Supplier
Library Robot	1	Roche
Computer for robot, installed with Director Software	1	
Bio-Rad C1000 Thermal Cycler or equivalent	1	User
Eppendorf 5810 Centrifuge	1	
V&P Scientific 24-Post Magnetic Bead Separation Block (magnet)	1	
Honeywell 1902h Barcode scanner	1	
Speedball 4126 Soft rubber brayer	1	

Labware	Quantity used	Supplier
Tecan Disposable troughs, 100 mL	2	Roche
Tecan Trough carrier, 100 mL, 3-Position	1	

Prepare to Create UNA

Confirm that the NAP file associated with the NAP barcode being processed has been successfully accessioned (imported). See Nucleic Acid Plate (NAP) prep and NAP File and Sample Data File.

Confirm that the AMB, R2B, and ETH reagents have been at room temperature for at least 30 minutes.

Visually inspect the reagent volume in each well of the AMB for liquid uniformity as evaporation could occur during storage.

If you notice an inconsistency with the vessel fill volume, see the AcfS User Guide, "Troubleshooting" for information on liquid dispensing problems.

IMPORTANT

Do not centrifuge AMB plates. If there is excess liquid on the plate seal, do not use that plate.

Record the lot number of AMB, R2B, and ETH for reagent lot tracking and trending.

IMPORTANT

Use reagents within 6 hours of removal from storage. Do not return the single use reagents (AMB) to storage after it has equilibrated. Doing so can affect reagent stability and lead to a failed test.

Obtain the room temperature DNA samples in the NAP plate.

Confirm that each sample in the 96-well, 0.8mL NAP contains 150 µL of DNA input.

Centrifuge the plates using the high-speed centrifuge at 1000 rcf for one minute to remove any condensation from the plates' seal.

Label a new Bio-Rad 96-well, hard shell PCR plate with a UNA barcode sticker (AD#-UNA) on the right side of the plate, opposite the lettered column. The barcode should be centered in the middle and flush with the bottom of the skirt and adhering to the recess of the skirt.

IMPORTANT

Do not allow the barcode to fold over the top or bottom of the plate (see Figure 8). Doing so can cause the plate to stick, leading to an instrument crash.

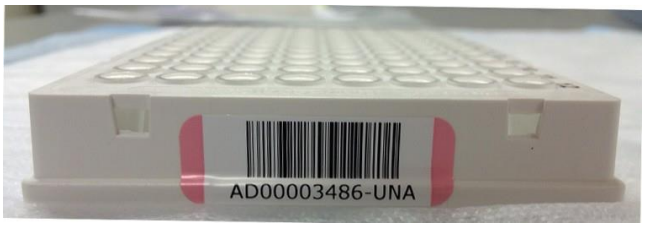

Figure 8. UNA Barcode Label Position

Label the 70% ethanol container with the ETH reagent barcode (AD#-ETH). Ensure that the barcode is unobstructed (Figure 9).

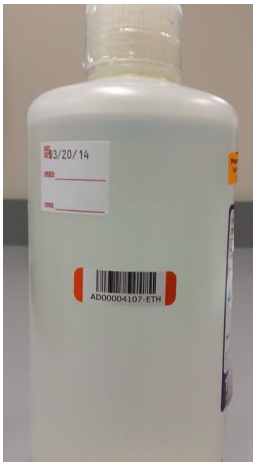

Figure 9. Ethanol Container with ETH Reagent Barcode

Prepare the Library Robot

⚠ WARNING

PHYSICAL INJURY HAZARD. Before starting any robotic step, ensure that the front safety shield of the Library Robot is closed. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument.

Prepare the Library Robot for operation. Refer to the *AcfS User Guide,* "Before operating the AcfS", for more details on robot preparation and loading.

Retrieve reagents and consumables needed for the Create UNA task. Refer to Table 10.

Under Library Tasks, select **Create UNA** under Library Tasks.

Obtain the R2B and ETH reagent bottles and mix the reagent by inverting the bottles three times.

Ensure that the 100 mL reagent troughs are marked with the liquid fill line for each reagent.

R2B is marked at 15 mL.

ETH is marked at 35 mL.

Label one of the 100 mL reagent troughs with the TD#-ETH barcode.

Label a second trough with the TD#-R2B barcode.

IMPORTANT

Barcoded troughs can be washed and reused for up to a month. Refer to Perform Cleanup.

Obtain a Tecan 100 mL trough carrier and place the barcoded and marked reagent troughs in the following order with the barcodes facing right (Figure 10).

R2B trough in Row 1.

ETH trough in Row 3.

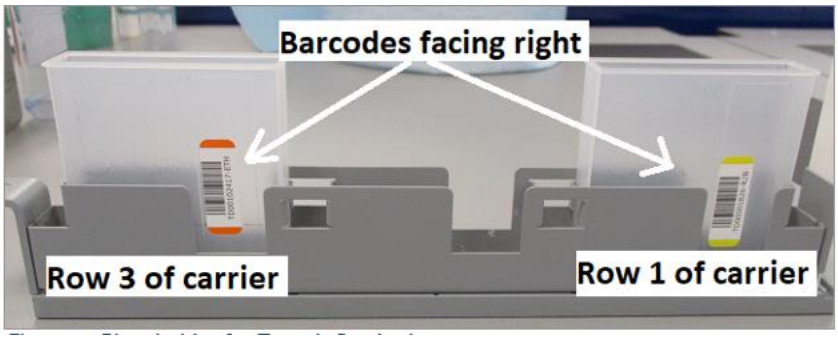

Figure 10. Trough Carrier and barcode placement

Remove the seal from prepared NAP in a gentle and continuous motion. Avoid shaking or lifting the plate.

Remove the seal from the AMB plate in a gentle and continuous motion. Avoid shaking or lifting the plates.

IMPORTANT

Check for adhesive residue on the AMB plate. Gently rub the adhesive off and ensure it is not tacky. Make sure to not shake/splash the plate while doing so. Residual adhesive can cause the plate to stick leading to an instrument crash.

Arrange all equipment, consumables and reagents on the Tecan carriers as specified by the Create UNA task worktable shown in Figure 11.

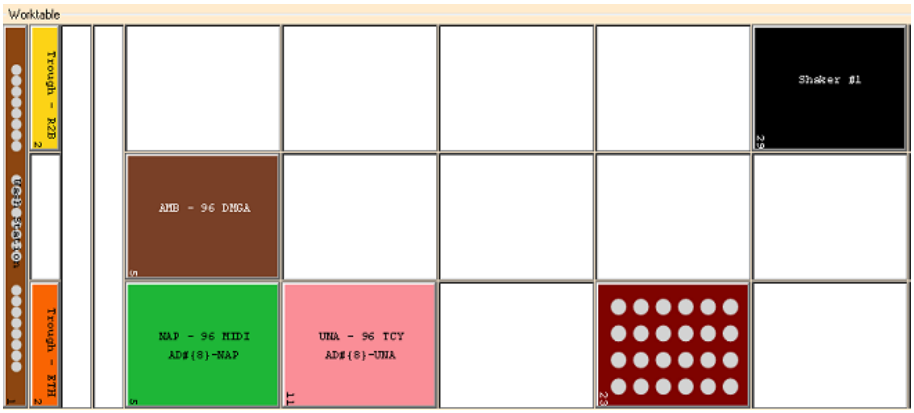

Figure 11. Create UNA Worktable

Confirm that the barcode on the 24-post Magnetic Bead Separation Block faces to the right (Figure 12).

Confirm that the reagent troughs and plates are barcoded and that the barcode is unobstructed and facing towards the right of the Library Robot (Figure 12).

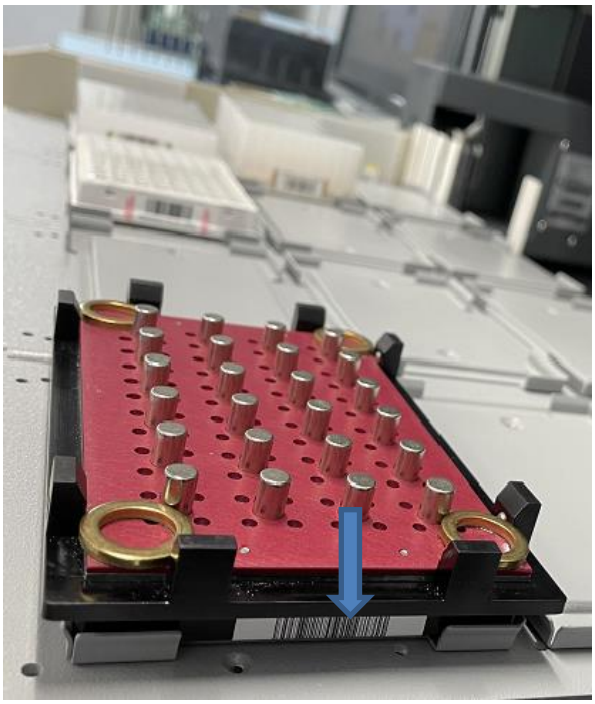

Figure 12. Barcode on the 24-post Magnetic Bead Separation Block

Confirm that all reagent plates are oriented with well A1 IS in the upper-left corner of the Tecan carrier and the plate seals are removed.

Confirm the NAP plate is in the proper orientation with the NAP barcode facing to the right and well A1 IS oriented to the upper-left corner of the Tecan carrier. Confirm the plate seal has been removed.

Confirm that the front safety shield to the Library Robot is closed.

Select **Run** in Director software.

Scan and Pour Reagents

Use a measurement device (such as a serological pipette or graduated cylinder) to measure liquids. **Take care not to spill reagents.** Refer to SDS if spill occurs (*SAFETY INFORMATION*).

Scan and Pour the R2B Reagent

In the Create UNA Task window, follow the prompt to scan the corresponding barcodes.

When prompted, slide the 100 mL trough carrier out from the deck and scan the R2B trough barcode.

Scan the R2B reagent bottle (AD#-R2B).

Pour 15 mL of the R2B reagent into the reagent trough up to the fill line.

Place the trough carrier back onto the deck.

Confirm that the front safety shield to the Library Robot is closed.

In Director software select **OK** to continue.

Scan and Pour the ETH Reagent

When prompted, slide the 100 mL trough carrier out from the deck and scan the ETH trough barcode.

Scan the ETH reagent container (AD#-ETH).

Pour 35 mL of the ETH reagent into the reagent trough up to the fill line.

Place the trough carrier back onto the deck, confirm that the front safety shield to the Library Robot is closed, then in Director software select **OK** to continue.

Seal and Inspect the UNA Plate and Check Plates

After completion of the Create UNA task, carefully remove the UNA plate from the Library Robot.

Use an adhesive seal and Speedball brayer to seal the UNA plate.

Make sure to thoroughly adhere the seal to the plate. Ensure that all edges of the seal are in contact with the plate. Evaporation during incubation can cause sample failures.

Visually check the UNA plate for liquid uniformity.

Remove the NAP plate from the deck of the Library Robot and visually check the residual volume for liquid uniformity.

Remove the AMB plate from the robot deck and visually check the residual volume for liquid uniformity. There should be less than 10 µL of beads remaining in each well.

If you notice an inconsistency with the vessel fill volume, refer to the AcfS User Guide, "Troubleshooting" for more information.

After you have checked the plates, incubate the UNA plate.

Run Inc1_UNA

Transfer the sealed UNA plate to a thermal cycler. On the thermal cycler, select the Inc1_UNA program to incubate the sealed UNA plate (Table 11).

Table 11. Thermal Cycler Program Inc1_UNA

Step	Temperature	Time
1	95°C	3
2	10°C	Forever

SAFE STOPPING POINT: If you do not plan on proceeding to the next task (Biotinylate UNA) directly after the completion of incubation, you can store the UNA plate at –20°C (-30°C to -15°C) for up to three days. Avoid repeatedly freezing and equilibrating the sample.

Perform Cleanup

While the UNA plate is incubating:

Refer to the AcfS User Guide, "After operating the AcfS" for details on how to prepare the Library Robot for the next task.

Thoroughly rinse the 100 mL troughs with deionized water and placed in a clean area to dry. It is recommended to replace the trough monthly.

Return back to Harmony IVD Kit 10100648001 and begin the Biotinylate UNA section of the Library Generation Tasks.

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GLOSSARY OF HARMONIZED SYMBOLS

	Temperature limit		Batch code (Lot)
	Use-by-date		Item number
	In vitro diagnostic medical device		Manufacturer
	Contains Sufficient for N tests		Consult Instructions For Use
	Date of Manufacture		Authorized representative in the European Community
	Do not dispose of electronic products in the general waste stream (Symbol used for Concerto Only)		Importer
	Physical hazard		ACMA standards compliance label (Symbol used for Concerto Only)
	Laser hazard		Unique Device Identification
	Radiation hazard		Explosion hazard
	Health Hazard Symbol		Shock hazard
	Corrosive hazard		

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Harmony IVD Kit

Concerto IVD Imager

TECHNICAL SUPPORT

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

The summary of safety and performance report can be found using the following link:
https://ec.europa.eu/tools/eudamed.

DOCUMENT REVISION

Document Revision Information	
Doc Rev. 2.0 06/2025	Added all consumables required but not supplied with the kit to Table 6. Corrected typographical errors that gave incorrect temperature values, e.g. period before 2 and missing negative sign. Corrected inconsistent lab storage location of TCP in Figure 1 and Figure 7. Clarified recommended number of system washes to be six for the Reagent Preparation task between reagent types and gave a recommended order of reagents to be prepped. Clarified that equivalent models of thermocyclers to Bio-Rad C1000 can be used as ancillary equipment. Removed references to step numbers that are no longer in document. Please contact your local Roche Representative if you have any questions.