Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow

Catalog Numbers 952380 and 952389

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow User Guide* (Pub. No. MAN0019172). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Introduction

Running the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow requires the following sets of steps:

1. Genomic DNA preparation, described in the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow User Guide* (Pub. No. MAN0019172).
2. Target preparation of the samples, performed according to the instructions in this document.
3. Array processing, described in the user guide.

This document describes manual target preparation, performed using the reagents and equipment described in the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide* (Pub. No. MAN0019173).

IMPORTANT! This quick reference contains an abbreviated set of instructions. Carefully read all the instructions in the user guide before running the target preparation method described here.

Note: An option for a 3-hour DNA precipitation step is available. See the user guide for details.

Assay notes

- This Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow allows you to run the Axiom™ PharmacoFocus™ Assay for 96 samples one time using 1 Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389).
- The mPCR preparation and GeneTitan™ reagent preparation steps require the use of disposable reservoirs with a "trough within a trough" design that maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.
- Remove seals from plates carefully and discard used seals. Do not reuse seals.
- Unless otherwise specified, all reagent modules are from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit. Stage 1A requires the QIAGEN™ Multiplex PCR *Plus* Kit (Cat. No. 206152) and is sufficient to process 96 samples. See the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow User Guide* (Pub. No. MAN0019172) for a complete list of equipment and consumables that are required for each stage.
- We recommend that you prepare the gDNA Sample Plate in a clean room. The clean room should be separate from the laboratory where the Axiom™ PharmacoFocus™ Assay is performed and be free of DNA amplified in other procedures.

Note: Axiom™ PharmacoFocus™ Mini 96-Array Plates require a total of 150 ng of gDNA. mPCR requires 50 ng and DNA amplification requires 100 ng.

Stage 1A: Perform multiplex PCR (mPCR)

Input required

The mPCR Sample Plate, with 10 µL of sample diluted to a concentration of 5 ng/µL in a 96-well PCR plate compatible with your thermal cycler.

Equipment, reagent, and sample plate handling

Quantity	Item	Details
Equipment		
1	Plate centrifuge at room temperature	
1	Approved thermal cycler[1] programmed with the **PharmacoFocus mPCR** protocol	**PharmacoFocus mPCR** protocol • 95°C for 5 minutes • 95°C for 30 seconds, 60°C for 180 seconds, and 72°C for 45 seconds—cycled 35 times • 68°C for 10 minutes • 4°C hold
Reagents from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)		
1 tube	◯ 10X Primer Mix	Thaw, vortex, briefly centrifuge, then keep on ice.
User supplied from the QIAGEN™ Multiplex PCR *Plus* Kit (100) (Cat. No. 206152).[2]		
3 tubes	2X Multiplex PCR Master Mix	Thaw, invert 10 times to thoroughly mix, briefly centrifuge, then keep on ice.
1 tube	5X Q-Solution	Thaw, vortex, briefly centrifuge, then keep on ice.
1 tube	RNase-free Water	Thaw, vortex, briefly centrifuge, then keep on ice.
mPCR Sample Plate		
Thaw the mPCR Sample Plate at room temperature. Vortex, centrifuge briefly, then place on cold 96-well metal chamber.		

[1] Ensure that the heated lid option is used and the thermal cycler is programmed to run in "9600 Mode" for the Applied Biosystems™ GeneAmp™ PCR System 9700, Veriti™ Thermal Cycler, and ProFlex™ System, and "Safe" mode for Eppendorf™ Mastercycler™ pro S.

[2] CoralLoad Dye in kit is not needed for the Axiom™ PharmacoFocus™ Assay.

Prepare the mPCR Master Mix

1. Label a 15-mL conical tube "mPCR".

2. To the mPCR tube, add the reagents that are listed in the following table and in the following order:
   a. Add water, Q-Solution, and the primers to the 15-mL conical tube, then cap the tube.
   b. Vortex and centrifuge briefly.
   c. Add 2,400 µL of the QIAGEN™ 2X Master Mix to the 15-mL conical tube.
      - It is necessary to use all 3 vials that are supplied by QIAGEN™.
      - Set a P1000 single-channel pipette to 800 µL, then remove this volume from the first vial.
      - Transfer the solution to the 15-mL conical tube. Change tips, then repeat this step for the remaining 2 vials.
   d. Mix thoroughly by gently inverting the tube end-over-end 10 times, then briefly centrifuge.

Reagent	One reaction	120 reactions
RNase-free Water	2 µL	240 µL
Q-Solution	4 µL	480 µL
10X mPCR Primer Mix	4 µL	480 µL
2X QIAGEN™ Multiplex PCR Master Mix	20 µL	2,400 µL
Total	**30 µL**	**3,600 µL**

3. Proceed immediately to set up the mPCR Reaction Plate.

Set up the mPCR Reaction Plate

1. Carefully pour the prepared mPCR Master Mix into a 25-mL reservoir.
2. Use a P200 multichannel pipette to carefully transfer 30 µL of mPCR Master Mix into the mPCR Sample Plate.
3. Seal the plate. Gently vortex, then centrifuge at 2,000 rpm for 30 seconds.
4. Load the plate onto the thermal cycler within 5 minutes.
5. Discard all leftover reagents.

Run the **PharmacoFocus mPCR** thermal cycler protocol

Note: The mPCR Sample Plate is now known as the mPCR Reaction Plate

1. Place mPCR Reaction Plate on thermal cycler, then run the **PharmacoFocus mPCR** protocol.
2. After the mPCR Reaction Plate has been placed on the thermal cycler, proceed to “Stage 1B: Amplify the genomic DNA” on page 4.

Store the mPCR Product Plate

Note: The mPCR Reaction Plate is now known as the mPCR Product Plate.

Do one of the following:

- For mPCR spike-in and fragmentation on another day, store the mPCR Product Plate at –20°C.
- For mPCR spike-in and fragmentation within 4 hours, store the mPCR Product Plate in a refrigerator (2–8°C).

Note: An mPCR QC gel can be run for qualitative evaluation of the mPCR reaction before the mPCR spike-in step during fragmentation. See the user guide for details.

Stage 1B: Amplify the genomic DNA

Input required

The Amplification Sample Plate, with 20 µL of each gDNA diluted to a concentration of 5 ng/µL in a 96-deepwell plate (ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate or Eppendorf™ DeepWell™ Plate 96, 2,000 µL).

Reagents required for Stage 1B

Reagent and cap color	Module
Reagents from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)	
Axiom™ 2.0 Denat Soln 10X	Module 1, –20°C (Part No. 901711)
Axiom™ 2.0 Neutral Soln	
Axiom™ 2.0 Amp Soln	
Axiom™ 2.0 Amp Enzyme	
Axiom™ Water	

Prepare for DNA amplification

1. Set the incubator or oven temperature to 37°C.
2. Set the centrifuge to room temperature.
3. Thaw the Amplification Sample Plate on the benchtop at room temperature, then vortex it, centrifuge it briefly, and leave it at room temperature.
4. Prepare reagents as indicated in the following table.

Reagent and cap color	Treatment
Axiom™ 2.0 Denat Soln 10X	Thaw, vortex, and centrifuge, then keep at room temperature.
Axiom™ 2.0 Neutral Soln	Thaw and vortex, then keep at room temperature.
Axiom™ 2.0 Amp Soln	Thaw and vortex, then keep at room temperature.
Axiom™ Water	Thaw and vortex, then keep at room temperature.
Axiom™ 2.0 Amp Enzyme[1]	Flick the tube 3 times, centrifuge it, then keep it in a –20°C cooler until ready to use. Just before using it, gently flick the tube 3 times to mix, then centrifuge it briefly.

[1] Leave at –20°C until ready to use.

Note: Allow ~1 hour for the Axiom™ 2.0 Amp Soln to thaw on the benchtop at room temperature. If the solution is not thawed after 1 hour, vortex it briefly and return it to the benchtop to complete thawing. The reagent bottles can also be thawed in a dish with ultra-pure water such as Millipore™ water. The Axiom™ 2.0 Amp Soln and the Axiom™ 2.0 Neutral Soln must be thoroughly mixed before use.

Prepare the Denaturation Master Mix

Carry out the following steps at room temperature.

1. To the 15-mL conical tube labeled “D MM”, add the amount of Axiom™ 2.0 Denat Soln 10X listed in the following table, then dilute it with the amount shown of Axiom™ Water from Module 1 (Part No. 901711).

Reagent and cap color	Per sample	Master mix 96+
Axiom™ 2.0 Denat Soln 10X	2 µL	400 µL
Axiom™ Water	18 µL	3.6 mL
Total volume	**20 µL**	**4.0 mL**

2. Vortex the tube, centrifuge briefly, then leave it at room temperature.

Add Denaturation Master Mix to samples

1. Gently pipet the Denaturation Master Mix using a P1000 or pour it into the reagent reservoir labeled “D MM”.
2. Add 20 µL of Denaturation Master Mix to each sample. Pipet directly into the liquid of each well. Do not mix by pipetting up and down.

   Note: This plate is now the Denaturation Plate.
3. Seal and vortex the Denaturation Plate. After vortexing, start the timer for a 10-minute incubation.
4. Centrifuge the Denaturation Plate in a room-temperature centrifuge for 1 minute at 1,000 rpm.

   Note: The centrifuge time is included in the 10-minute incubation.
5. After incubation, immediately add the Axiom™ 2.0 Neutral Soln.

Add Axiom™ 2.0 Neutral Soln to samples

1. Pour the Axiom™ 2.0 Neutral Soln into a reagent reservoir labeled “N Soln”.
2. Add 130 µL of Axiom™ 2.0 Neutral Soln to each sample. Pipet down the wall of each well. Do not mix by pipetting up and down.

   Note: The Denaturation Plate is now the Neutralization Plate.
3. Seal the Neutralization Plate, vortex it, then briefly centrifuge it.
4. Proceed immediately to Amplification Master Mix preparation.

Prepare the Amplification Master Mix

1. Pipet the amount of Axiom™ 2.0 Amp Soln listed in the following table into a 50-mL tube labeled "Amp MM".

Reagent and cap color	Per sample	Master mix 96+
Axiom™ 2.0 Amp Soln	225 µL	26.0 mL
Axiom™ 2.0 Amp Enzyme	5 µL	578 µL
Total volume	**230 µL**	**26.58 mL**

2. Vortex the Amplification Master Mix well, invert the tube 2 times, then vortex it again.

Add Amplification Master Mix to samples

1. Slowly pour the Amplification Master Mix into the reagent reservoir labeled “Amp MM”.
2. Slowly add 230 µL Amplification Master Mix to each well of the Neutralization Plate. Pipet down the wall of the well. Do not mix by pipetting up and down.

   Note: The Neutralization Plate is now the Amplification Plate.
3. Blot the top of the plate with a laboratory tissue. Seal the plate tightly, vortex twice, then centrifuge for 1 minute at 1,000 rpm.
4. Place the sealed Amplification Plate in an oven set at 37°C, then leave it undisturbed for 23 ±1 hours.

Freeze the plate or proceed

After the incubation finishes, do one of the following:

- Proceed to “Stage 2: Fragment and precipitate the DNA” on page 6.
- Store the Amplification Plate at –20°C.

Note: If freezing, do not perform the stop amplification reaction step before you store the Amplification Plate at –20°C. The stop amplification reaction step is performed after thawing the frozen plate. See “Prepare for fragmentation and precipitation” on page 6.

Stage 2: Fragment and precipitate the DNA

Input required

The mPCR Product Plate and the Amplification Plate from Stage 1 in a 96-deepwell plate (ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate or Eppendorf™ DeepWell™ Plate 96).

Reagents required for Stage 2

Reagent and cap color	Module
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)	
Axiom™ Frag Enzyme (leave at –20°C until ready to use)	Module 2-1, –20°C (Part No. 901528)
Axiom™ 10X Frag Buffer	
Axiom™ Precip Soln 2	
Axiom™ Frag Diluent	Module 2-2, 2–8°C (Part No. 901529)
Axiom™ Frag Rxn Stop	
Axiom™ Precip Soln 1	
User-supplied	
Isopropanol, 99.5%, 70 mL	—

Prepare for fragmentation and precipitation

Note: If the Amplification Plate or the mPCR Product Plate was frozen at the end of Stage 1, thaw the plates before beginning Stage 2. See instructions in Chapter 2 of the user guide for notes on thawing and centrifuging prior to changing the seal to avoid cross-contamination.

Set oven and centrifuge temperatures

1. Set up 2 incubators or ovens. Set one oven at 37°C and one at 65°C.
2. Set the centrifuge to room temperature.

Perform mPCR spike-in to the Amplification Plate

IMPORTANT! Ensure that the mPCR Product Plate has been thoroughly mixed before spike-in to the Amplification Plate.

1. Vortex the mPCR Product Plate, then centrifuge at 1,000 rpm for 30 seconds.
2. Transfer 10 µL of the mPCR products into the corresponding well of the Amplification Plate. Pipet up and down a few times to ensure complete liquid transfer from the pipette tip.
3. Seal the plate. Ensure that the seal is securely attached to the plate to minimize evaporation during the next steps.
4. Thoroughly mix by vortexing the plate for 30 seconds, then centrifuge briefly.
5. Immediately proceed to the next step (“Stop the DNA amplification reaction” on page 6).

Stop the DNA amplification reaction

1. Place the Amplification Plate in the 65°C oven.
2. Incubate for 20 minutes.

3. Prepare reagents at the start of the 65°C incubation of the Amplification Plate.

Reagent and cap color	Module	Handling instructions
Axiom™ 10X Frag Buffer	2-1	Thaw, vortex, and keep on ice.
Axiom™ Frag Enzyme	2-1	Flick tube 3X, briefly centrifuge, then keep in –20°C cooler until ready to use.
Axiom™ Precip Soln 2	2-1	Thaw, vortex, briefly centrifuge, then keep at room temperature.
Axiom™ Frag Diluent	2-2	Vortex, briefly centrifuge, then keep on ice.
Axiom™ Frag Rxn Stop	2-2	Vortex, then keep at room temperature.
Axiom™ Precip Soln 1	2-2	Vortex, then keep at room temperature.
Isopropanol, 99.5%, 70 mL	N/A	Keep at room temperature.

4. Transfer the sample plate from the 65°C oven to the 37°C oven and incubate for 45 minutes.

Prepare the Fragmentation Master Mix

Start making the Fragmentation Master Mix (Frag MM) 5 minutes before completion of the 37°C incubation.

1. Add the reagents listed in the following table, in the order shown, to a 15-mL tube.
   a. Add the Axiom™ Frag Enzyme to the Fragmentation Master Mix at the end of the 45-minute 37°C incubation.

Reagent and cap color	Per sample	Master mix 96+
Axiom™ 10X Frag Buffer	45.7 µL	6.0 mL
Axiom™ Frag Diluent	10.3 µL	1.35 mL
Axiom™ Frag Enzyme	1.0 µL	131 µL
Total volume	**57 µL**	**7.48 mL**

2. Vortex the Frag MM tube twice.
3. Slowly pour the Fragmentation Master Mix into a reagent reservoir labeled “Frag MM”. Place the reservoir at room temperature.

Add Fragmentation Master Mix to samples

IMPORTANT! Work quickly to perform this set of steps to minimize the time that the Fragmentation Plate is out of the 37°C oven.

1. Carefully remove the Amplification Plate from the 37°C oven and place it on the benchtop at room temperature.

   Do *not* place the Amplification Plate on ice.

2. Add 57 µL of Fragmentation Master Mix to each reaction. Pipet directly into the liquid of each well. Do *not* mix by pipetting up and down.

   Note: The Amplification Plate is now the Fragmentation Plate.

3. Seal the Fragmentation Plate, then vortex it twice.
4. Start the timer for 30 minutes.
5. Briefly centrifuge the Fragmentation Plate at room temperature.
6. Quickly transfer the plate to the 37°C oven, then incubate for 30 minutes.

CAUTION! Watch for the end of the 30-minute incubation. *Fragmentation is an exact 30-minute incubation step.* Longer or shorter incubation times can lead to poor performance of the assay.

7. Prepare the Axiom™ Frag Rxn Stop solution a few minutes before the end of the 30-minute incubation.

Add the Axiom™ Frag Rxn Stop solution to the Fragmentation Plate

1. A few minutes before the end of the 30-minute incubation, pour the Axiom™ Frag Rxn Stop solution into a reagent reservoir labeled “Stop”.
2. At the end of the 30-minute fragmentation incubation, remove the Fragmentation Plate from the oven. Place it on the benchtop at room temperature.
3. End the fragmentation reaction by adding 19 µL of Axiom™ Frag Rxn Stop to each reaction. Pipette directly into the liquid of each well. Do not mix by pipetting up and down.
4. Seal the Fragmentation Plate, then vortex and briefly centrifuge it at 1,000 rpm.
5. Leave the Fragmentation Plate on the benchtop while you prepare the Precipitation Master Mix.

Prepare the Precipitation Master Mix

Carry out the following steps at room temperature.

1. Prepare the Precipitation Master Mix (Precip MM) by adding 218 µL of Axiom™ Precip Soln 2 directly to the Axiom™ Precip Soln 1 bottle.

Reagent and cap color	Per sample	Master mix 96+
Axiom™ Precip Soln 1	238 µL	26 mL
Axiom™ Precip Soln 2	2 µL	218 µL
Total volume	**240 µL**	**26.22 mL**

2. Vortex the Precip MM bottle. Place it on the benchtop at room temperature.

Add Precipitation Master Mix to samples

Carry out the following steps at room temperature.

1. Pour the Precipitation Master Mix into a reagent reservoir labeled “Precip MM”.
2. Add 240 µL Precipitation Master Mix to each sample. Rest the pipette tip against the wall of the well while delivering. Do not mix by pipetting up and down.

   Note: The Fragmentation Plate is now the Precipitation Plate.
3. Seal the Precipitation Plate, vortex, then briefly centrifuge.

Prepare and add isopropanol to the Precipitation Plate

1. Remove the Precipitation Plate from the centrifuge. Place it on the benchtop at room temperature.
2. Pour 65 mL of isopropanol into a 100-mL reagent reservoir labeled “ISO”.
3. Add 600-µL of isopropanol to each sample, then mix well by pipetting up and down 6–7 times. Observe the solution in the tips. It should look homogeneous after pipetting 6–7 times. If not, repeat mixing a few more times until the solution looks homogeneous. Do not vortex the plate after adding the isopropanol to avoid cross-contamination of the samples.
4. Blot the top of the plate with a laboratory tissue, then seal tightly with MicroAmp™ Clear Adhesive Film.

Freeze the Precipitation Plate

Designate a shelf in a –20°C freezer where the plates can be left undisturbed. In addition, the freezer must not be subjected to frequent temperature excursions.

1. Carefully transfer the Precipitation Plate into the –20°C freezer.
2. Incubate the Precipitation Plate for the desired length of time, either overnight (16–24 hours) or 3 hours.
3. After the incubation finishes, proceed to “Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC” on page 10

   Note: The shortened 3-hour precipitation allows you to proceed to “Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC” on page 10 followed by “Stage 4: Transfer, denature, then hybridize the denatured samples” on page 14 on day 2 of the assay workflow.

Store the mPCR Product Plate

Store the mPCR Product Plate at –20°C for optional QC.

Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC

CAUTION! Some steps in this stage must be performed under a fume hood.

IMPORTANT! For troubleshooting and support purposes, we strongly recommend that you perform the gel QC and OD quantification process controls after resuspension.

Input required

The Precipitation Plate from Stage 2: Fragment and precipitate the DNA.

Reagents required for Stages 3A, 3B, and 3C

Reagent and cap color	Module
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)	
Axiom™ Hyb Buffer	Module 2-1, –20°C (Part No. 901528)
Axiom™ Hyb Soln 1	
Axiom™ Resusp Buffer	Module 2-2, 2–8°C (Part No. 901529)
Axiom™ Hyb Soln 2	
Other reagents and gel required for QC steps (optional)	
Gel Diluent, 100-fold dilution of TrackIt™ Cyan/Orange Loading Buffer	—
E-Gel™ *48* Agarose Gels, 4%	—
Applied Biosystems™ 25 bp DNA Ladder (Cat. No. 931343) or a similar product prepared as instructed by manufacturer.	—
Nuclease-free water, ultrapure MB Grade (for OD QC Plate and Dilution QC Plate preparation)	—

Reagent preparation for Stages 3A, 3B, and 3C

Thaw and prepare reagents according to the following table.

Reagent and cap color	Preparation
Axiom™ Hyb Buffer	Vortex, then keep at room temperature.
Axiom™ Hyb Soln 1	Thaw, vortex, centrifuge, then keep at room temperature.
Axiom™ Hyb Soln 2	Vortex, centrifuge, then keep at room temperature.
Axiom™ Resusp Buffer	Warm to room temperature (1 hour). Vortex before use.

Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellet

Centrifuge the Precipitation Plate and dry the DNA pellets

CAUTION! During this step, handle the plate gently to avoid disturbing the pellets. Do not bump or bang the plate against another object.

1. Preheat the oven to 37°C.
2. Transfer the Precipitation Plate from the –20°C freezer to a pre-chilled centrifuge.
3. Centrifuge the plate for 40 minutes at 4°C at 3,200 x *g*.
4. Immediately after the 40-minute centrifugation time, empty the liquid from the plate using the following steps:
   a. Carefully remove the seal from the Precipitation Plate. Discard the seal.

b. Invert the plate over a clean waste container. Allow the liquid to drain. Collect the liquid, then discard it according to local, state, and federal regulations.

c. While still inverted, gently press the plate on a pile of laboratory tissues on a bench. Allow it to drain for 5 minutes. Transfer the plate to a new pile of tissues twice during the 5-minute drain.

5. Turn the plate right side up and place it in an oven for 20 minutes at 37°C to dry.

6. Seal the plate tightly.

7. Do one of the following:

   - Proceed directly to the next stage. See “Stage 3B: Resuspend the pellets and prepare for hybridization” on page 11. Leave the sample plate at room temperature.
   - Tightly seal the Precipitation Plate, then store at –20°C.

Stage 3B: Resuspend the pellets and prepare for hybridization

Prepare for resuspension and hybridization

Set the centrifuge to room temperature.

Add the Axiom™ Resusp Buffer to the DNA pellets

A plate stored at –20°C after drying the pellets must be allowed to sit at room temperature for 1.5 hours before carrying out resuspension.

Ensure that the Axiom™ Resusp Buffer has equilibrated to room temperature before adding it to the dry pellets in step 2.

Carry out the following steps at room temperature.

1. Pour the Axiom™ Resusp Buffer into a reagent reservoir labeled “Resus”.

2. Transfer 35 µL of Axiom™ Resusp Buffer to each well of the Precipitation Plate. Avoid touching the pellets with the pipette tips.

   Note: The Precipitation Plate is now the Resuspension Plate.

3. Seal the plate tightly.

   Note: Blue pellets should be visible at the bottom of the wells.

Resuspend the DNA pellets

1. Place the sealed Resuspension Plate on one of the following shakers, then run for the time that is specified.

   - Thermo Scientific™ Compact Digital Microplate Shaker: 900 rpm for 10 minutes
   - Jitterbug™: Speed 7 for 10 minutes

2. Inspect the Resuspension Plate from the bottom. If the pellets are not dissolved, repeat step 1.

3. Centrifuge the plate briefly at 1,000 rpm.

Prepare the Hybridization Master Mix

CAUTION! Perform the remainder of Stage 3B under a fume hood.

1. While the Resuspension Plate is shaking, prepare the Hybridization Master Mix in a 15-mL tube.
2. Add the reagents as indicated in the following table, in the order shown, to the 15-mL tube labeled “Hyb MM”.

Reagent and cap color	Per sample	Master mix 96+
Axiom™ Hyb Buffer	70.5 µL	7.8 mL
Axiom™ Hyb Soln 1	0.5 µL	55.6 µL
Axiom™ Hyb Soln 2	9 µL	1.0 mL
Total	**80 µL**	**8.86 mL**

3. Vortex the tube twice to mix.

Prepare the Hyb-Ready Plate

1. Select a 96-well plate that is compatible with the thermal cycler model that is used for sample denaturation.
2. Label the 96-well PCR plate “Hyb Ready [Plate ID]”.
3. Transfer the entire contents of each well of the Resuspension Plate to the corresponding wells of the Hyb-Ready Plate.
4. Pour the Hybridization Master Mix into a reagent reservoir labeled “Hyb MM”.
5. Add 80 µL of the Hybridization Master Mix to each well of the Hyb-Ready Plate.

   Note: Change pipette tips after each addition.
6. Seal the Hyb-Ready Plate, vortex it twice, then centrifuge it briefly.

Freeze or proceed

Do one of the following:

- Proceed to “Stage 3C: Perform quantification and fragmentation QC checks” on page 12. We strongly recommend performing the QC check.
- Proceed to “Stage 4: Transfer, denature, then hybridize the denatured samples” on page 14.
- Store the Hyb-Ready Plate at –20°C.

Stage 3C: Perform quantification and fragmentation QC checks

Before proceeding to “Stage 4: Transfer, denature, then hybridize the denatured samples” on page 14, we highly recommend that you perform quantification and fragmentation quality control checks.

Prepare for sample QC

Prepare the reagents

Obtain the following reagents for sample QC.

- Nuclease-free water, 15 mL, for the water reservoir
- Gel diluent, 15 mL
- 25 bp DNA Ladder prepared as instructed by the manufacturer.
- Two E-Gel™ *48* Agarose Gels, 4%

Perform QC checks

1. Prepare the Dilution QC Plate and the OD QC Plate.
    a. Pour 15 mL of Nuclease-free Water into a reagent reservoir.
    b. Add 33 µL of Nuclease-free Water to each well of the Dilution QC Plate.
    c. Add 90 µL of Nuclease-free Water to each well of the OD QC Plate (96-well UV-Star™ Plate).
2. Prepare the Dilution QC Plate.
    a. Transfer 3 µL of the hybridization-ready sample from each well of the Hyb-Ready Plate to the corresponding well of the Dilution QC Plate.
    b. Seal, vortex, then briefly centrifuge the plate.
3. Prepare the OD QC Plate.
    a. Transfer 10 µL of each Dilution QC Plate sample to the corresponding wells of the OD QC Plate and mix by pipetting up and down.
4. Prepare the Gel QC Plate.
    a. Pour 15 mL of gel diluent into a reagent reservoir.
    b. Add 120 µL of gel diluent to each well of the Gel QC Plate.
    c. Transfer 3 µL of each Dilution QC Plate sample to the corresponding wells of the Gel QC Plate.
    d. Seal, vortex, then briefly centrifuge the plate.
5. Run the gel.
    a. Tightly seal the Gel QC Plate, vortex, and briefly centrifuge.
    b. Onto a 4% agarose e-gel load:
        - 15 µL from each well of the Gel QC Plate.
        - 15 µL of 25 bp DNA Ladder into the marker wells. Follow the product instructions for dilution method.
        - 15 µL of water to any unused wells.
    c. Run for 19 minutes.

Freeze or proceed

Do one of the following:

- Proceed to “Stage 4: Transfer, denature, then hybridize the denatured samples” on page 14.
- Store the Hyb-Ready Plate at –20°C.

Stage 4: Transfer, denature, then hybridize the denatured samples

CAUTION! Parts of this stage must be performed under a fume hood.

Input required

The Hyb-Ready Plate from Stage 3.

Equipment required for Stage 4

Quantity	Item
1	GeneTitan™ MC Instrument
1	Rainin™ P200 12-channel pipette
As needed	Pipette tips
1	Thermal cycler, programmed with **PharmacoFocus Denature** protocol. Use the heated lid option when setting up or running the protocol. • 95°C for 10 minutes • 48°C for 3 minutes • 48°C hold
1	Hyb-Ready Denaturation Plate, 96-well PCR plate, one of the following: • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601) for Eppendorf™ Mastercycler™ pro S • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641) for Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, and Applied Biosystems™ ProFlex™ System
1	96-well metal chamber warmed in a 48°C oven[1]
1	Axiom™ PharmacoFocus™ Mini 96-Array Plate
1	384-Layout GeneTitan™ Hybridization Tray[2]

[1] The 96-well metal chamber coming out of a 48°C oven is warm to the touch. Use gloves or mitts if the metal chamber feels too hot.

[2] The Consumables for the GeneTitan™ MC Instrument are packaged separately from the array plates. The 384-Layout GeneTitan™ Hybridization Tray, along with other GeneTitan™ consumables, are included in the Axiom™ 384HT GeneTitan™ High Volume Consumables Kit (Cat. No. 902629) or Axiom™ GeneTitan™ 384HT Consumables Kit (Cat. No. 952385).

Reagents required and handling for Stage 4

Reagent	Module	Handling instructions
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit		
Axiom™ Wash Buffer A	Module 3, Room temperature	Invert 2-3 times for mixing before filling GeneTitan™ bottle
Axiom™ Wash Buffer B		Invert 2-3 times for mixing before filling GeneTitan™ bottle
Axiom™ Water		—

Prepare for transfer, denaturation, and hybridization

1. Preheat the 96-well metal chamber in a 48°C oven.
2. Allow the array plate to equilibrate to room temperature for at least 25 minutes.
   a. Leave the array plate in the pouch at room temperature, for at least 25 minutes to allow the plate to come to room temperature, before opening and loading in the GeneTitan™ MC Instrument.
   b. At the end of the array warm up time, open the pouch and scan the array plate barcode into the GeneTitan™ Array Plate Registration file.

WARNING! Do not remove the array plate from the protective base or touch the surface of any of the arrays.

3. Power on the thermal cycler to prepare for the **PharmacoFocus Denature** protocol to run with the heated lid option selected.

Prepare hybridization-ready samples stored at –20°C

1. Warm the Hyb-Ready Plate at room temperature for 5 minutes. It is not necessary to equilibrate the plate longer than 5 minutes.
2. Check the Hyb-Ready Plate to ensure that it is sealed well. If the plate is not sealed well:
   a. Centrifuge the plate, then carefully remove the old seal.
   b. Wipe any condensation from the top of the plate, then gently blot dry with a laboratory tissue.
   c. Tightly reseal the plate using a fresh seal.
3. Vortex the Hyb-Ready Plate for 30 seconds to mix, then centrifuge at 1,000 rpm for 30 seconds.
   It is important that the samples are thoroughly mixed prior to transfer to the Hyb-Ready Denaturation Plate.
4. Place the Hyb-Ready Plate at room temperature.

Transfer samples from the Hyb-Ready Plate to the Hyb-Ready Denaturation Plate

1. Ensure that the Hyb-Ready Plate from Stage 3 is tightly sealed.
2. Vortex the plate, then centrifuge at 1,000 rpm for 30 seconds.
3. Label a new Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted plate (or appropriate PCR plate) "Hyb-Ready Denaturation Plate".
   Use the PCR plate type that is compatible with the thermal cycler that will be used for denaturation.
4. Obtain a P200 12-channel pipette, then set it at 50 µL.
5. Transfer 50 µL of hybridization-ready sample solution from the Hyb-Ready Plate to the Hyb-Ready Denaturation Plate.
6. Tightly seal the Hyb-Ready Denaturation Plate.
   This Hyb-Ready Denaturation Plate can be stored at –20°C if the denaturation step will be performed later.

After preparing the Hyb-Ready Denaturation Plate, approximately 62 µL of sample remains in the Hyb-Ready Plate. Carefully seal the Hyb-Ready Plate and store at –20°C. After the assay is successfully completed, the plate can be discarded.

Prepare the GeneTitan™ MC Instrument

Before you denature the Hyb-Ready Denaturation Plate samples, ensure that the GeneTitan™ MC Instrument is ready for use. The following is a brief summary of the steps to perform.

1. Launch the GeneChip™ Command Console™ software, then select **GCC GeneTitan Control**.
2. From the Launcher window, open **GCC Portal ▸ Samples ▸ GeneTitan™ Array Plate Registration**.
3. Upload the GeneTitan™ Array Plate Registration file.
   If you do not upload your registration file before scanning the array plate barcode, the software assigns default names to your samples.
   Note: When creating the GeneTitan™ Array Plate Registration file, you can add the barcode of the hybridization tray as a sample file attribute. Adding the barcode as an attribute enables traceability in the system. See the *GeneChip™ Command Console™ User Guide*, for details on adding attributes to sample files.
4. Select the **System Setup** tab.
5. For **Setup Option**, select **Hyb-Wash-Scan**.
6. Click **Next**.
7. Complete the following in the **Plate information** section:
   a. **Barcode**: Scan or manually enter the array plate barcode, then click **Next**.
   b. **Protocol Name**: Select the protocol name, then click **Next**.
8. Fill the Wash A, Wash B, and Rinse bottles with Axiom™ Wash Buffer A, Axiom™ Wash Buffer B, and Axiom™ Water, respectively.

9. Empty the Waste bottle.
10. Press the blue confirmation button on the GeneTitan™ MC Instrument to continue.
11. Open the trash bin and empty, then press the blue confirmation button to continue.
12. Remove used trays and plates when drawers open, then press the blue confirmation button to continue.

Denature the hybridization-ready samples on the Hyb-Ready Denaturation Plate

1. Ensure that the thermal cycler is powered on and that the **PharmacoFocus Denature** protocol with the heated lid option is selected.
2. Open the lid of the thermal cycler, then place the sealed Hyb-Ready Denaturation Plate on the thermal cycler.
   Check the integrity of the seal as evaporation during denaturation can negatively impact assay performance.
3. Close the lid.
   For thermal cyclers with variable lid tension, follow manufacturer's instructions for adjusting lid tension.
4. Start the **PharmacoFocus Denature** protocol.

IMPORTANT! Avoid leaving denatured samples at room temperature for any length of time. When you are ready to transfer the denatured samples to the hybridization tray at the end of the **PharmacoFocus Denature** protocol, place the plate on a 96-well metal chamber preheated at 48°C to minimize sample cooling.

Prepare the hybridization tray and load into the GeneTitan™ MC Instrument

If needed, review the proper procedures for working with and labeling GeneTitan™ trays in *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow User Guide*.

CAUTION! Perform the following steps under a fume hood.

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1. Remove the 384-Layout GeneTitan™ Hybridization Tray from packaging.
2. Label the hybridization tray.

   IMPORTANT! It is critical that you write only on the proper location of the hybridization tray, on the edge in front of wells A1 and F1.
3. After the **PharmacoFocus Denature** protocol has completed, remove the Hyb-Ready Denaturation Plate from the thermal cycler, then place it into a 96-well metal chamber that has been prewarmed in an oven at 48°C.
4. Move the warm 96-well metal chamber containing the Hyb-Ready Denaturation Plate to a fume hood.
5. Remove the seal from Hyb-Ready Denaturation Plate, then discard.
6. Place the hybridization tray under the fume hood, then remove the hybridization tray cover.
7. Obtain a P200 12-channel pipette and set it at 35 µL.

8. Slowly transfer the denatured samples from the wells of the 96-array format Hyb-Ready Denaturation Plate into the corresponding wells in quadrant 1 of the 384-Layout GeneTitan™ Hybridization Tray, using the guidance for plate format switching listed in the following table.

96-array format Hyb-Ready Denaturation Plate	384-Layout GeneTitan™ Hybridization Tray
Row A	Row A, odd wells
Row B	Row C, odd wells
Row C	Row E, odd wells
Row D	Row G, odd wells
Row E	Row I, odd wells
Row F	Row K, odd wells
Row G	Row M, odd wells
Row H	Row O, odd wells

   - Dispense to the first stop to avoid creating bubbles.
   - Change pipette tips after each transfer; discard the tip even if it shows some volume left.
   - Ensure that there are no air bubbles present in the hybridization tray. Puncture any air bubbles that you see using a clean pipette tip.
   - There is no need to spread the sample around the bottom of the hybridization tray wells. Sample distribution across the well occurs when the array plate is stacked together with the hybridization tray by the GeneTitan™ MC Instrument.

9. Load the array plate and hybridization tray into the GeneTitan™ MC Instrument.

IMPORTANT! After the GeneTitan™ MC Instrument has stacked the array plate and hybridization tray, the instrument extends the drawer. The sandwich of the array plate and hybridization tray must be manually clamped and inspected before the array processing can begin. Carefully review and execute the array plate/hybridization tray clamping procedure steps.

Hybridization continues on the GeneTitan™ MC Instrument for 23.5 to 24 hours before loading the Ligation/Staining/Stabilization reagent trays into the instrument occurs. Near the end of the 23.5- to 24-hour hybridization period in the GeneTitan™ MC Instrument, see “Stage 5: Prepare the GeneTitan™ reagents and trays” on page 19.

Plate format switching

Plate format switching is performed during the hybridization transfer step. The denatured, hybridization-ready samples are transferred from a 96-well PCR plate (Hyb-Ready Denaturation Plate) to a 384-Layout GeneTitan™ Hybridization Tray. The samples must only be transferred to the quadrant 1 wells of the hybridization tray. The following figure illustrates the hybridization transfer step when the switch from 96-well format to 384-well format occurs.

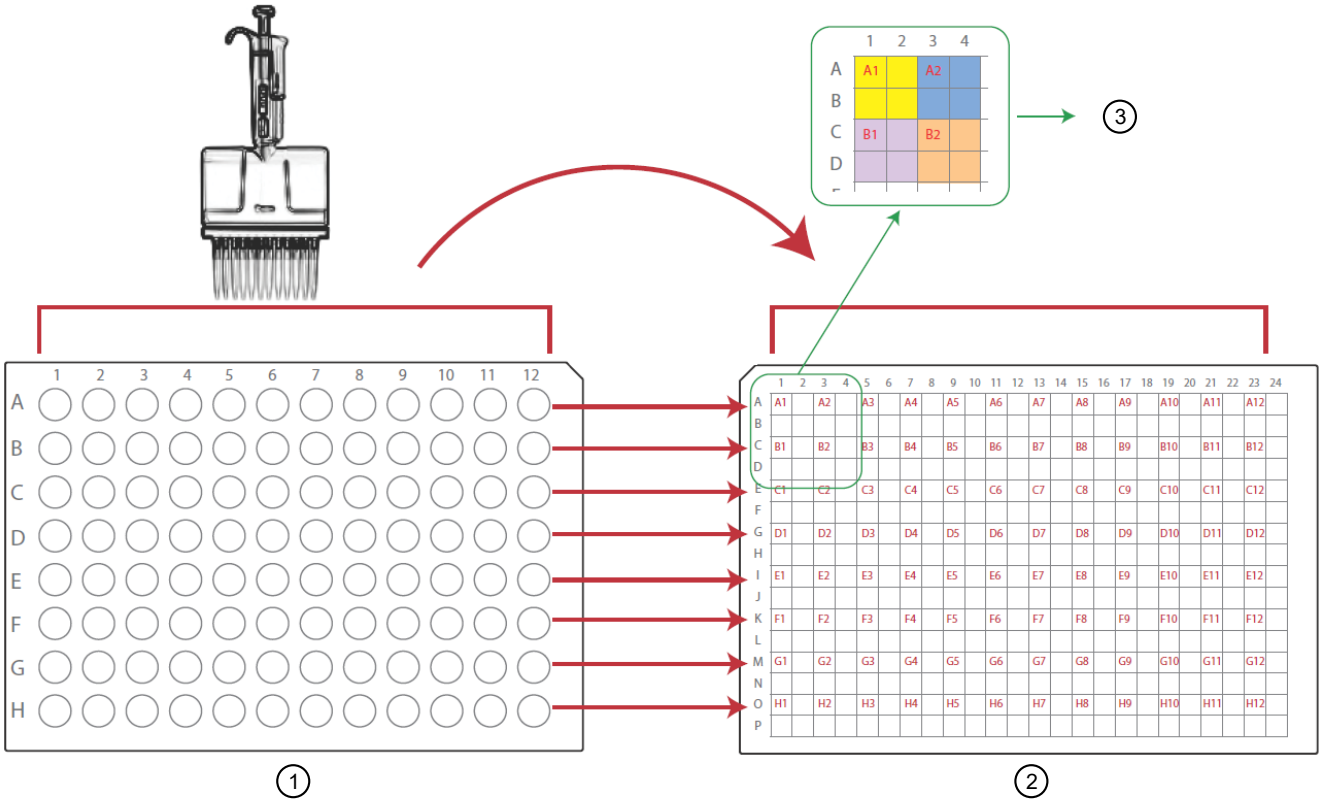

Figure 1 Plate format switching during hybridization transfer.

① 96-format Hyb-Ready Denaturation Plate

② 384-Layout GeneTitan™ Hybridization Tray

③ Quadrants explained: A 384-format plate consists of 96 four-well sections (96 x 4 = 384). Each of the 4 well in a section is a "quadrant". In the example shown, wells A1, A3, C1, and C3 of the of the 384-format plate are all designated as Quadrant 1 (Q1) wells within their respective 4-well section.

The red well numbers in the 384-Layout GeneTitan™ Hybridization Tray image represent the corresponding well transferred from the 96-well Hyb-Ready Denaturation Plate (all in Quadrant 1—the upper left quadrant of a section).

Stage 5: Prepare the GeneTitan™ reagents and trays

Reagents required and reagent handling for Stage 5

Prepare reagents according to the following table.

Module	Qty	Reagent and cap color	Thaw at room temp, then place on ice	Place on ice	Place at room temp	Handling instructions
Module 4-1 –20°C	1	Axiom™ Ligate Buffer[1]			✓	Vortex for 30 seconds. [1]
	1	Axiom™ Ligate Enzyme	⊗ Do not thaw. Keep at –20°C until ready to use.			**Immediately before use:** Gently flick tube 3 times, then centrifuge briefly.
	1	Axiom™ Ligate Soln 1	✓			Vortex, then centrifuge briefly.
	1	Axiom™ Probe Mix 1	✓			Vortex, then centrifuge briefly.
	1	Axiom™ Stain Buffer	✓			Vortex, then centrifuge briefly.
	1	Axiom™ Stabilize Soln	✓			Vortex, then centrifuge briefly.
Module 4-2 2°C to 8°C	1	Axiom™ Ligate Soln 2			✓	Vortex, then centrifuge briefly.
	1	Axiom™ Probe Mix 2[2]		✓		Gently flick tube 3 times, then centrifuge briefly.
	1	Axiom™ Wash A[1]			✓	Vortex for 30 seconds.[1]
	1	Axiom™ Stain 1-A[2]		✓		Gently flick tube 3 times, then centrifuge briefly.
	1	Axiom™ Stain 1-B[2]		✓		Gently flick tube 3 times, then centrifuge briefly.
	1	Axiom™ Stain 2-A[2]		✓		Gently flick tube 3 times, then centrifuge briefly.
	1	Axiom™ Stain 2-B[2]		✓		Gently flick tube 3 times, then centrifuge briefly.
	1	Axiom™ Stabilize Diluent[1]		✓		Vortex, then centrifuge briefly.[1]
	1	Axiom™ Water			✓	Vortex.
	1	Axiom™ Hold Buffer[2]			✓	Vortex for 30 seconds. Pour in reservoir.
Estimated reagent thawing time is 30 minutes.						

[1] Check for precipitate. If precipitate is present, repeat the vortex and centrifuge step.
[2] These solutions are light sensitive. Keep tubes out of direct light for a prolonged length of time.

Guidelines for handling reagents with precipitates

When the Axiom™ PharmacoFocus™ Module 4-2 is stored at 4°C, precipitation in the form of clear crystals can sometimes occur in the Wash A and Stabilize Diluent. See information in the user guide about handling reagents with precipitates for instructions to ensure that any precipitate is returned to solution before use.

Note: The presence of some precipitate does not adversely impact assay performance.

Prepare the stain, ligation, and stabilization master mixes

Prepare the Stain 1 Master Mix

1. Add reagents to a 15-mL conical tube labeled "S1" in the following order. This recipe provides enough for both S1 reagent trays.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Wash A	76.8 µL	8.8 mL
Axiom™ Stain Buffer	1.6 µL	184 µL
Axiom™ Stain 1-A	0.8 µL	92 µL
Axiom™ Stain 1-B	0.8 µL	92 µL
Total	**80 µL (40 µL x 2)**	**9.2 mL**

2. Gently invert the tube 10 times to mix. Do not vortex.
3. Place the tube on ice and protect it from direct light.

Prepare the Stain 2 Master Mix

1. Add reagents to a 15-mL conical tube labeled "S2" in the following order.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Wash A	38.4 µL	4.6 mL
Axiom™ Stain Buffer	0.8 µL	96 µL
Axiom™ Stain 2-A	0.4 µL	48 µL
Axiom™ Stain 2-B	0.4 µL	48 µL
Total	**40 µL**	**4.8 mL**

2. Gently invert the tube 10 times to mix. Do not vortex.
3. Place on ice, then protect from direct light.

Prepare the Stabilization Master Mix

1. Add reagents to a 15-mL conical tube labeled "Stbl" in the following order.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Water	35.5 µL	4.3 mL
Axiom™ Stabilize Diluent	4.0 µL	480 µL
Axiom™ Stabilize Soln	0.5 µL	60 µL
Total	**40 µL**	**4.8 mL**

2. Vortex the tube at high speed for 3 seconds. Place it on ice.

Prepare the Ligation Master Mix—part 1

The Ligation Master Mix is prepared in 2 parts.

1. Add reagents to a 15-mL conical tube labeled “Lig” in the following order.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Ligate Buffer	25.2 µL	3.1 mL
Axiom™ Ligate Soln 1	5.0 µL	624 µL
Axiom™ Ligate Soln 2	1.2 µL	150 µL
Total	**31.4 µL**	**3.9 mL**

2. Mix well by vortexing the Lig tube for 3 seconds, then plate it on ice.

Prepare the Ligation Master Mix—part 2

The Ligation Master Mix is prepared in 2 parts.

1. Remove the Axiom™ Ligate Enzyme from the –20°C freezer. Place it in a cooler chilled to –20°C.

2. Add reagents to a 15-mL conical tube labeled "Lig" in the following order.

   Note: Gently flick the Axiom™ Ligate Enzyme tube 2-3 times, then centrifuge it briefly immediately before adding the enzyme to the master mix.

Reagent and cap color	Per array	Master mix 96+
Ligation Master Mix from part 1 preparation	31.4 µL	3.9 mL
Axiom™ Probe Mix 1	4.0 µL	499 µL
Axiom™ Probe Mix 2	4.0 µL	499 µL
Axiom™ Ligate Enzyme	0.6 µL	75 µL
Total	**40 µL**	**5.0 mL**

3. Gently invert the tube 10 times to mix. Do not vortex.

4. Place the tube on ice and protect it from direct light.

Aliquot master mixes and Axiom™ Hold Buffer into trays

Label the GeneTitan™ reagent trays

IMPORTANT! It is critical that you write only on the proper location of the stain/reagent trays, on the edge in front of wells A1 to F1.

Obtain the 384-layout stain trays and label each specific tray as indicated in the following table.

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan™ MC Instrument, the front of each tray must be marked in a way that identifies its contents.

Stain tray type	Label color	Label the tray
384-Layout GeneTitan™ Stain Tray (Stain 1-1)	White	Stain 1-1
384-Layout GeneTitan™ Stain Tray (Stain 1-2)	White	Stain 1-2
384-Layout Axiom™ Stain 2 Tray	Blue	Stain 2
384-Layout Axiom™ Ligation Tray	Yellow	Lig
384-Layout Axiom™ Stabilization Tray	Green	Stbl

IMPORTANT! Always aliquot reagents to the bottom of the reagent tray. Avoid touching the sides or the top of the wells with the pipette tips. Droplets close to or on the top of the well dividers can cause the cover to stick to the tray during GeneTitan™ MC Instrument processing.

Place trays on the benchtop to pipet. If the trays will not be used immediately, protect them from light by covering with foil or placing them in a cabinet.

When aliquoting ligation, staining, and stabilization reagents to the trays, it is not necessary to spread the reagent to each corner of the well. The reagent spreads evenly when the array plate is inserted into the reagent tray during processing with the GeneTitan™ MC Instrument.

Scan tray: It is important to fill all 96 wells with Axiom™ Hold Buffer. The scan tray has an open-bottom design, so it is very important that all 96 wells of the scan tray receive 170 µL of Axiom™ Hold Buffer.

Stain trays: Fill only quadrant 1 of the stain trays with ligation, staining, and stabilization reagents.

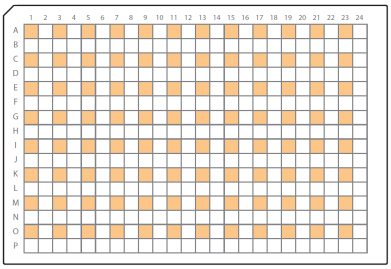

Figure 2 Quadrant 1 wells of a 384-layout Axiom™ stain tray.

Aliquot the Stain 1 Master Mix

1. Pour the S1 Master Mix into a reagent reservoir labeled “S1”.
2. Aliquot 40 µL per well to both trays labeled “S1”. Dispense to the first stop only to avoid creating bubbles.
3. If bubbles are present, puncture them with a pipette tip.
4. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place covers on the S1 trays. Orient the covers correctly on the trays with the notched corners together.
6. Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Stain 2 Master Mix

1. Pour the S2 Master Mix into a reagent reservoir labeled “S2”.
2. Aliquot 40 µL per Q1 well to the tray labeled “S2”. Dispense to the first stop only to avoid creating bubbles.
3. If bubbles are present, puncture them with a pipette tip.
4. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the S2 tray. Orient the cover correctly on the tray with the notched corners together.
6. Protect the tray from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Stabilization Master Mix

1. Pour the Stabilization Master Mix into a reagent reservoir labeled “Stbl”.
2. Aliquot 40 µL per Q1 well to the tray labeled "Stbl". Dispense to the first stop only to avoid creating bubbles.
3. If bubbles are present, puncture them with a pipette tip.
4. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the tray. Orient the cover correctly on the tray with the notched corners together.

Aliquot the Ligation Master Mix

1. Pour the Ligation Master Mix into a reagent reservoir labeled “Lig”.
2. Aliquot 40 µL per Q1 well to the tray labeled “Lig”. Dispense to the first stop only to avoid creating bubbles.
3. If bubbles are present, puncture them with a pipette tip.
4. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the Lig tray. Orient the cover correctly on the tray with the notched corners together.
6. Protect the tray from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Axiom™ Hold Buffer to the scan tray

1. Vortex the Axiom™ Hold Buffer, then pour it into a reagent reservoir labeled “Hold”.
2. Remove the scan tray from its pouch.
3. Remove the scan tray cover but leave the scan tray on its protective blue base.
4. Aliquot 170 µL of Axiom™ Hold Buffer to *every* well of the 96-plate scan tray. Dispense to the first stop and avoid touching the bottom of the tray.
5. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
6. Cover the tray by orienting the notched corner of the scan tray cover over the notched edge of the tray with the flat side of the cover against the scan tray.

Limited product warranty

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Products:
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Products:
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Axiom™ PharmacoFocus™ Mini 96-Array Plate Core

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

The information in this guide is subject to change without notice.