

Axiom™ 384HT and Mini 96 gDNA Sample Preparation

Pub. No. MAN0017719 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Introduction

Running the Axiom™ 2.0 Assay for 384 and mini 96-array format samples requires the following sets of steps:

1. Preparation of genomic DNA, as described in this document.
2. Target preparation of the samples, as described in one of the following quick reference documents:
 - *Axiom™ 2.0 Assay 384HT Array Format Automated Workflow Quick Reference—Biomek™ FXP* (Pub. No. 703270)
 - *Axiom™ 2.0 Assay 384HT Array Format Automated Workflow Quick Reference—Biomek™ i7 Method V1.0* (Pub. No. MAN0017557)
 - *Axiom™ 2.0 Assay Mini 96-Array Format Manual Workflow Quick Reference* (Pub. No. 703436)
3. Array processing, as described in *GeneTitan™ MC Protocol for Axiom™ 384HT Array Plate Processing Quick Reference* (Pub. No. MAN0017596).

IMPORTANT! This document contains an abbreviated set of instructions. Read all the instructions in Chapter 2, *Genomic DNA preparation and requirements* of the appropriate user guide for more details on the protocol and sample requirements.

- *Axiom™ 2.0 Assay 384HT Array Format Automated Workflow User Guide—Biomek™ FXP* (Pub. No. 703268)
- *Axiom™ 2.0 Assay 384HT Array Format Automated Workflow User Guide—Biomek™ i7* (Pub. No. MAN0017555)
- *Axiom™ 2.0 Assay Mini 96-Array Format Manual Workflow User Guide* (Pub. No. 703434)

Requirements

Review this information before proceeding with the DNA amplification for the automated target preparation.

The genomic DNA (gDNA) you process must meet the following requirements:

- Starting DNA must be double-stranded for accurate concentration determination.
- DNA must be of high purity and be free of DNA polymerase inhibitors.
- DNA must not be degraded.

Table 1 Genomic DNA sample input requirements

Sample type	Volume per well	Input mass per well	gDNA concentration
Human	8.7 µL	100 ng	11.5 ng/µL
Diploid plants and animals	8.7 µL	150 ng	17.2 ng/µL
Polyploid plants and animals	8.7 µL	200 ng	23.0 ng/µL

IMPORTANT! Prepare your genomic DNA sample plate in a clean room. The clean room should be separate from the laboratory where the assay is performed and must be free of DNA amplified in other procedures.

Note: Use at least one positive control DNA sample on each plate. For human samples, Genomic DNA Standard (Ref 103) (Cat. No. 951957) can serve as the control. For plant or animal samples, use a genomic DNA sample that meets the specifications that are mentioned above, is from the same species that is represented on the array, and ideally has passed previously in the Axiom™ Genotyping Solution. If no DNA control for your human sample type is available, then the Genomic DNA Standard (Ref 103) can serve as a positive control during target preparation.

Reagents required

Reagent
Genomic DNA Standard (Ref 103) (positive control for human samples), Cat. No. 951957, -20°C
Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) Cat. No. 75793
Positive control gDNA (if genotyping non-human samples)

Equipment and consumables required

Quantity	Item
As required	Adhesive seals for plates
1	Ice bucket, filled with ice
1 each	Pipettes: single channel P10 or P20 Optional: multichannel P10 or P20
As required	Pipette tips
1	Plate, deepwell: - For the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow Axygen™ 384 well clear V-bottom 240 µL polypropylene deep-well non-treated plate, sterile (Cat. No. P-384-240SQ-C-S)^[1] - For the Axiom™ 2.0 Assay Mini 96-Array Format Manual Workflow, one of the following: - Eppendorf™ 96 deepwell, 2,000 µL (Cat. No. 951033481)^[2] - Square 96 Deepwell Plate, 2 mL^[3]
1	Plate centrifuge
1	Microtiter plate fluorimeter Quant-iT™ PicoGreen™ dsDNA Assay Kit (required only if no concentration measurements available for samples)
1	Vortexer

^[1] This plate is part of the Axiom™ 384HT Consumables Kit for Biomek™ FX[®] (Cat. No. 902288), and the Axiom™ 384HT Consumables Kit for Biomek™ i7 (Cat. No. 905424). If purchased directly from the supplier, a unique barcode must be added.

^[2] This plate is part of the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (Cat. No. 902986).

^[3] This plate is part of the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit v2 (Cat. No. 952431).

(ABgene™ 96-well 2.2 mL Polypropylene Deepwell Storage Plate, Cat. No. AB0932)

Prepare genomic DNA samples

1. Prepare gDNA control sample.

- If genotyping human samples, use the Genomic DNA Standard (Ref 103) (Cat. No. 951957).
- If genotyping non-human samples:
 - Select a sample to be the positive control. Ensure that it is double-stranded, highly pure, is free of DNA polymerase inhibitors, and is not degraded. Ideally, this sample has shown passing performance when used in the Axiom™ Genotyping Assay.
 - Dilute the sample to a working concentration. For diploid plants and animals, dilute the sample to 17.2 ng/µL with reduced EDTA TE buffer in a sterile, DNase-free tube. For polyploid plants and animals, dilute the sample to 23.0 ng/µL. Prepare sufficient material for the entire study. We recommend one positive control per array plate.

2. Thaw samples and control.

Thaw the Genomic DNA Standard (Ref 103) and positive control sample to temperature. To thaw, either:

- Place items on benchtop for one hour.

- Thaw in a water bath:
 - Fill a small plastic dish with ultra-pure water (such as Millipore™ water). Do not overflow. The level of the water must not overflow when the sample tubes or plates are placed in the bath.
 - Place the DNA samples in the water bath and thaw for 30 minutes.
 - Wipe water off the sample plate before removing the seal to avoid contamination of the samples.

3. Quantify, then dilute gDNA.

- Gently vortex (50% maximum), then centrifuge samples.
- Quantify each sample. The Quant-iT™ PicoGreen™ dsDNA Assay Kit is recommended.
- Using reduced EDTA TE buffer, dilute each sample to a concentration of:
 - 11.5 ng/µL for human DNA samples
 - 17.2 ng/µL for diploid plant and animal DNA samples
 - 23 ng/µL for polyploid plant and animal DNA samples
- Seal, vortex, then centrifuge.

4. Aliquot the diluted samples and the control.

Aliquot diluted samples and reference positive control DNA to the deep well plate:

- Aliquot 8.7 µL of each diluted gDNA sample (the equivalent of 100–200 ng of gDNA depending on the final sample type to be hybridized).
- Aliquot 8.7 µL of the positive control sample. We recommend including at least one positive control on each plate.
- Seal, then centrifuge.

5. Freeze or proceed. At this point you can:

- Store the sample plate at –20°C, or
- Proceed to DNA amplification for target preparation.

Note: If proceeding immediately to DNA amplification, you can leave the gDNA sample plate at room temperature.

6. Create a GeneTitan™ Array Plate Registration file.

Note: It is important to create and upload a GeneTitan™ Array Plate Registration file with your sample information before loading the array plate and hybridization tray in the GeneTitan™ Instrument. Create (but not upload) this file at the same time you prepare your plate of genomic DNA. When your samples are ready for hybridization, scan the array plate barcode and upload the file to GeneChip™ Command Console™ (GCC).

GeneTitan™ Array Plate Registration file contains information that is critical for:

- Data file generation during imaging.
- Tracking the experimental results for each sample that is loaded onto an array plate.

Detailed instructions for creating this file are in the appropriate assay user guide:

- *Axiom™ 2.0 Assay 384HT Array Format Automated Workflow User Guide—Biomek™ FX^P* (Pub. No. 703268)
- *Axiom™ 2.0 Assay 384HT Array Format Automated Workflow User Guide—Biomek™ i7* (Pub. No. MAN0017555)
- *Axiom™ 2.0 Assay Mini 96-Array Format Manual Workflow User Guide* (Pub. No. 703434)

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

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

Limited product warranty

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

Products:
Axiom™ 2.0 384HT Reagent Kit
Axiom™ 2.0 Assay Mini 96 Reagent Kit



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

Products:
Axiom™ 384HT Array Plate
Axiom™ Mini 96-Array Plate

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Revision	Date	Description
B.0	15 January 2021	Information added for the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit v2.
A.0	12 September 2018	Initial release in Thermo Fisher Scientific document control system. Supersedes legacy Affymetrix publication number 703163. Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty. Updated to reflect that Axiom Reference gDNA 103 has been removed from the reagent kit and has been made available for purchase separately.

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