TrueMark™ PGx Panel, 384–well Plate

Catalog Number A40003215

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *TrueMark™ PGx Panel, 384–well Plate User Guide* (Pub. No. MAN0030150). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference applies only to fixed panels.

For TrueMark™ PGx Custom Plating, see *TrueMark™ PGx Panel, 384–well Plate User Guide* (Pub. No. MAN0030150).

Overview of sample preparation

Before beginning the procedure, collect samples and isolate the DNA. For DNA isolation procedures, see *TrueMark™ PGx Panel, 384–well Plate User Guide* (Pub. No. MAN0030150).

Prepare reactions for fixed panels

For TrueMark™ PGx Custom Plating, see *TrueMark™ PGx Panel, 384–well Plate User Guide* (Pub. No. MAN0030150).

1 Prepare a DNA stock solution

IMPORTANT! UV absorbance measurements and fluorometric analysis can overestimate the quantity of DNA from buccal swabs. These methods cannot differentiate human DNA and microbiome DNA in the sample.

1. Mix the samples well, especially if the samples were stored.
2. Quantify DNA by any of the following methods.
   - Real-time PCR with a TERT reference assay (recommended)
   - UV absorbance measurements
   - Fluorometric analysis
3. Prepare a 4 ng/µL DNA stock solution in nuclease-free water for the number of reactions required, plus 10% overage (10.0 ng of input DNA per reaction).

   4 ng/µL is the recommended concentration.

2 Set up the PCR reactions: 64 assay format

Before you perform PCR, a 0.2-mL, 96-well plate is used to combine the sample (or NTC) with the master mix. A maximum of six samples can be used with plates in the 64 assay format. To have sufficient volume for the reactions, each sample (or NTC) occupies two wells of the plate. The following example, where each sample occupies two adjacent wells, is appropriate for a fixed channel pipette.

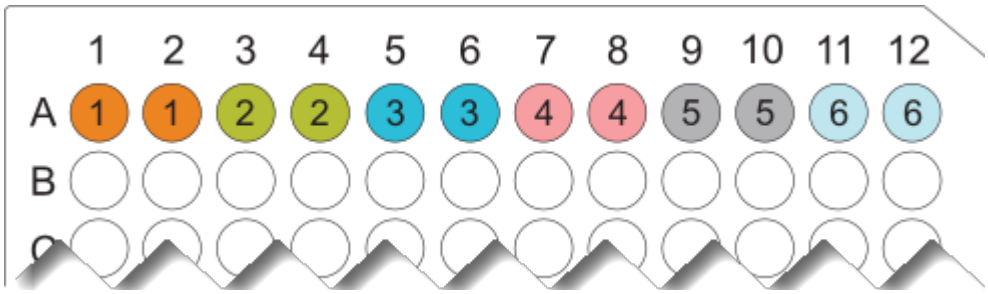

Figure 1 Example layout of a 96-well plate for the 64 assay format Numbers in wells designate a sample, or an NTC.

1. Add 90 µL of TaqPath™ ProAmp™ Master Mix to each designated well of a 96-well plate.

   For example, add the master mix to each well of row A of the plate.

   Note: Transfer the TaqPath™ ProAmp™ Master Mix to a reservoir to use a multichannel pipette for this step.

2. Add 90 µL of each sample (or NTC) to the designated wells of the plate, as shown in Figure 1.

   For example, sample 1 is added to wells A1 and A2, sample 2 is added to wells A3 and A4, and so on.

3. Seal the plate with adhesive foil, then vortex briefly to mix the contents.

4. Centrifuge the plate at 500 × *g* for 1 minute to collect the contents at the bottom of each well.

3 Transfer reactions to the TrueMark™ PGx Panel, 384–well Plate: 64 assay format

Each 384-well plate (64 assay format) can be used to test up to 6 samples. Prepare the number of plates that are required for your samples.

1. Remove the foil wrap and heat seal from a 384-well plate.

2. Transfer 5 µL of the reaction mix for samples 1–6 (see page 2) from the sample plate into the appropriate wells of the 384-well plate (Figure 2 on page 3).

   Note: We strongly recommend using an electronic 12-channel pipette to simplify this portion of the workflow.

3 *(continued)*

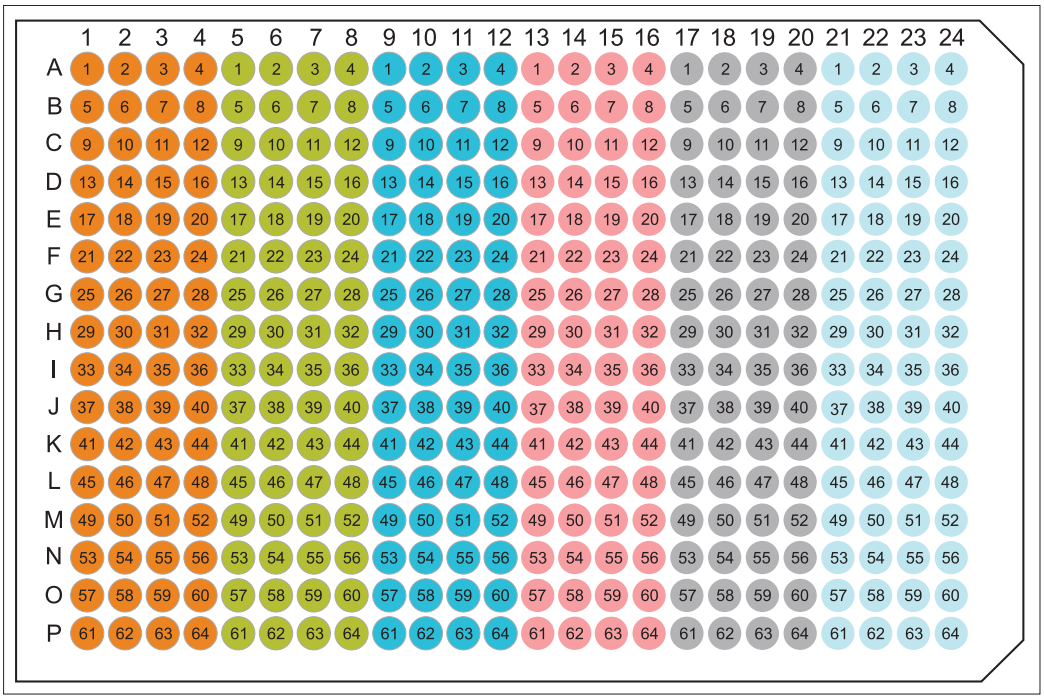

Figure 2 384-well plate layout: 64 assay format

The numbers in the well represent the pre-plated assays (1–32). Colors represent individual samples.

1. Sample 1
2. Sample 2
3. Sample 3
4. Sample 4
5. Sample 5
6. Sample 6

3. Seal the plate well, then vortex to mix the contents.

   The TaqMan™ Copy Number Assays are in rows H and I of the plate. These assays require sufficient mixing.

4. Centrifuge the plate at 500 × *g* for 1 minute to collect the contents at the bottom of each well.

5. Repeat for the remaining samples.

Perform real-time PCR on TrueMark™ PGx Panels, 384–well Plates

1 Create an experiment template (EDT)

1. Create a run on the instrument software or the instrument with the EDT file that is included with the plates.

2. Confirm the properties.

Property	Setting
Block	384-well
Experiment type	Genotyping
Reagents	TaqMan™ Reagents
Instrument run	Standard Include: Pre-PCR Read, Amplification, Post-PCR Read
Sample volume	5 µL

1 *(continued)*

3. Confirm the thermal cycling parameters.

Step	Temperature	Ramp rate	Time	Cycles
Pre-Read	60°C	1.6°C per second	30 seconds	1
Enzyme activation	95°C		10 minutes	1
Denaturation Annealing/Extension	95°C 60°C		15 seconds 60 seconds	50
Post-Read	60°C		30 seconds	1 (Hold)

4. Assign targets.

   The targets are assigned for fixed plates.

   To export results from Diomni™ Design and Analysis (RUO) Software 3 for additional analysis with AlleleTyper™ Software, the targets for copy number variation analysis must have the suffix ***_cn***.

5. Assign samples.

   For fixed plates, the plate file contains generic sample names, for example, ***Sample 1***, ***Sample 2***. The sample assignment in the plate file can be edited to contain more specific sample information.

6. Save the updated plate file.

Save the plate file that contains the target assignments and the sample names.

You can also save the plate file that the target assignments, but not the specific sample names.

Saving the plate file with the sample names depends on the workflow of your laboratory.

The plate file can be reused for multiple runs.

2 **Set up the real-time PCR instrument and start the run**

Each 384-well plate requires a unique plate file.

For detailed instructions about how to set up and run the real-time PCR instrument, see the documentation for the instrument or the data collection software.

1. Load the plate onto the instrument.
2. Start the run on the instrument.

   Use the EDT file that was created (see "Create an experiment template (EDT)" on page 3).

Analyze data

For more information, see the documentation for Diomni™ Design and Analysis (RUO) Software 3.

1 **Transfer data files**

1. On the instrument or the computer that is running the data collection software, download or export the EDS files.
2. Transfer the files to a location that can be accessed by Diomni™ Design and Analysis (RUO) Software 3.

2 **Set up a project**

1. Create a new project.
2. Add data files to the project.
3. *(Optional)* Define the project.

   The following items are defined:
   - Targets
   - SNPs
   - CNVs

2 *(continued)*

- Samples

4. Add the Assay Information File (AIF) to the project.
5. Add the CNV assay conversion to the project.

   A CNV assay conversion that is applicable to the fixed panels is included in the system project template.

3 **Analyze genotyping data**

1. View the calls.

   Automatic calls are the default method used by the software.
2. *(Optional)* Perform manual calls.
3. *(Optional)* Use a classification scheme to make calls.
4. View the call rates.
5. Export the data for further analysis.

   Data can be exported for analysis with AlleleTyper™ Software.

4 **Analyze copy number variation data**

1. View the predicted copy numbers.

   The calculated copy number can be viewed if the predicted copy number is not reported.
2. View the quality metrics.
3. Export the data for further analysis.

   Data can be exported for analysis with AlleleTyper™ Software.

Limited product warranty

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

Revision history: Pub. No. MAN0030152 B

Revision	Date	Description
B	2 April 2026	Nuclease-free water was specified as the diluent for preparation of the DNA stock solution (“Prepare a DNA stock solution” on page 1).
A	8 April 2025	New document for the TrueMark™ PGx Panel, 384–well Plate.