Novallele™ Genotyping Assay User Manual

For Novallele™ Genotyping Assays, Novallele™ Genotyping Mastermix and 2× Novallele™ Oligo Dilution Buffer

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

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Company Info

Canon BioMedical, Inc. is focused on empowering the biomedical research and healthcare communities by developing, manufacturing and marketing innovative technologies and solutions. The technologies and solutions developed will help enable clinicians and scientists to improve our health and advance science. Canon BioMedical will continue to pursue innovative solutions in line with Canon's Kyosei philosophy through the use of existing and emerging Canon technology, as well as strategic partnerships.

Kyosei - defined as "all people, regardless of race, religion, or culture, harmoniously living and working together into the future."

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Contents of Products

This user manual applies to three types of products (as defined below) that are ordered independently. The tables below describe the contents of each product package. The Novallele Genotyping Mastermix, a PCR chemistry formulation allowing rapid PCR followed by High Resolution Melt Analysis (HRMA), may be used with either Novallele Genotyping Assays or with user-designed assays that will require the use of 2× Novallele Oligo Dilution Buffer. **The use of Novallele Genotyping Mastermix is essential for successful function of the Novallele Genotyping Assays. Do not replace this reagent with other commercial products**.

Product Name	Novallele Genotyping Mastermix
Catalog Number	40018
Number of reactions	166 - 10μl reactions
Volume of reagent and packaging	600 μl in one vial
Documentation included	Product Specification Sheet

Product Name	Novallele Genotyping Assays
Catalog Number	Depends on the human SNP selected
Number of reactions	166 - 10μl reactions
Volume of reagent and packaging	600 μl in one vial
Documentation included	Product Specification Sheet with assay-specific protocol

Product Name	2× Novallele Oligo Dilution Buffer
Catalog Number	40017
Number of reactions	Makes enough Assay for 166 - 10μl reactions
Volume of reagent and packaging	300 μl in one vial
Documentation included	Product Specification Sheet

Shipping and Storage

Novallele Genotyping Assays are shipped under environmental conditions. Novallele Genotyping Assays should be stored at 2-8°C. When stored correctly, this product is stable for six (6) months from the date of receipt.
Novallele Genotyping Mastermix is shipped under environmental conditions. Novallele Genotyping Mastermix should be stored at 2-8°C. When stored correctly, this product is stable for six (6) months from the date of receipt.
2× Novallele Oligo Dilution Buffer is shipped under environmental conditions. 2× Novallele Oligo Dilution Buffer should be stored at 2-8°C. When stored correctly, this product is stable for six (6) months from the date of receipt.

If the products arrive damaged or without cold ice packs, please call Canon BioMedical Support at 1-844-CANON-BIO.

All of the Novallele reagents mentioned above can be stored during test preparation for up to two (2) hours at room temperature (15- 25°C).

Intended Use – RESEARCH USE ONLY

All Novallele Genotyping Assays, Novallele Genotyping Mastermix and 2× Novallele Oligo Dilution Buffer are For Research Use Only. Not for use in diagnostic procedures.

Product Warranty

Canon BioMedical warrants that each Novallele Genotyping Assay, Novallele Genotyping Mastermix or 2× Novallele Oligo Dilution Buffer, each a "Novallele Product", sold to the Customer will substantially perform in accordance with the written specifications therefor set forth in the applicable package insert or user manual originally included by Canon BioMedical with each Novallele Product delivered to the Customer until the instructed "use by" date set forth on the Novallele Product packaging. Canon BioMedical's sole liability for, and the Customer's sole remedy, under this limited warranty will be for Canon BioMedical to replace the nonconforming Novallele Product with a conforming Novallele Product, provided that in each case Canon BioMedical is notified and returned by the Customer during the warranty period of the nonconforming Novallele Product as instructed by an authorized Canon BioMedical employee. The warranty period on any replacement Novallele Product will be the period from the shipment date until the replacement Novallele Product "use by" date.

A copy of the Canon BioMedical Inc. standard terms and conditions applicable to the Novallele Genotyping Assays, Novallele Genotyping Mastermix and 2× Novallele Oligo Dilution Buffer can be obtained on request. If you have questions about product specifications or performance, please call Canon BioMedical Support at 1-844-CANON-BIO or visit www.canon-biomedical.com

Technical Assistance

At Canon BioMedical, we strive to deliver the highest quality technical support. Our support team is composed of experienced scientists with extensive academic and industry laboratory expertise in genotyping and PCR technologies. If you have any questions about Canon BioMedical products, please do not hesitate to contact us.

For technical assistance and more information, please visit our website at www.canon-biomedical.com or call Canon BioMedical at 1-844-CANON-BIO.

SAFETY INFORMATION

When working in a laboratory, use personal protective equipment (including lab coat, disposable gloves, and protective eyewear). Follow your local institutional policies and procedures regarding the appropriate handling of biological materials and lab reagents. For more information, please consult the appropriate safety data sheets (SDSs). To obtain a SDS for Canon BioMedical products, please contact Canon BioMedical Support at 1-844-CANON-BIO.

Introduction

Humans share almost the same sequence of 3 billion DNA bases within our 23 pairs of chromosomes. Current estimates indicate that up to 0.1% of human genome DNA may vary, meaning any two unrelated individuals may differ at fewer than 3 million DNA positions. The importance of these small variations in our genomes stems from their ability to influence disease risk, response to therapeutics, and even predict how we look and behave. A Single Nucleotide Polymorphism (SNP) is a type of variation within the genome occurring commonly within a population in which a single nucleotide — A, T, C or G — in the genome differs between members of a biological species or paired chromosomes. SNPs – also commonly known as mutations- are one of the most important types of genetic changes influencing common diseases, typical genetic variations include insertions, deletions and copy number variations (CNVs).

A diverse set of genotyping technologies including sequencing, microarrays, in situ hybridization and PCR-based methods are used to detect mutations. Such variety of methods enables researchers to match the correct technology with not only their experimental question, but also user preferences for data analysis, cost and turnaround time.

Principles and Procedure

Novallele Genotyping Assays in combination with Novallele Genotyping Mastermix contain all the reagents needed to identify specific SNPs or mutations within a given human genomic DNA sample.

The Novallele Genotyping Assays and Novallele Genotyping Mastermix perform rapid PCR. HRMA of the PCR products follows the completion of rapid PCR. First, DNA samples provided by the end-user are mixed with the Novallele Genotyping Mastermix and Novallele Genotyping Assay in either a microcentrifuge tube or the well of a PCR plate. Subsequent amplification of DNA by PCR, and amplicon melting is performed rapidly. Finally, changes in the fluorescence intensity of the amplicons are captured by the optical system within the user's HRMA-compatible thermocycler. HRMA is a method for detecting changes in DNA sequence. It uses a fluorescent dye at saturating concentrations that will bind to double-stranded DNA during the PCR reaction. Once the PCR is complete, the reaction temperature is raised, double-stranded DNA denatures, and the bound dye is released, decreasing the fluorescence. The exact fluorescence change with increasing temperature will depend on the DNA sequence of the amplicon. A shift in melting temperature (Tm) or a change in the shape of the melt curve is observed when a variation is detected in a sample DNA compared to reference DNA sample.

Equipment and Reagents to be supplied by User

In addition to the Novallele Genotyping Assays or the 2× Novallele Oligo Dilution Buffer, and Novallele Genotyping Mastermix, the following reagents and equipment are required:

 96-well or 384-well PCR plate with Optical Thin-Wall 8-Cap Strips or Optical Adhesive Film or Rotor-Disc with Rotor-Disc Heat-Sealing Film or the appropriate thermocycler compliant reaction vessels (such as PCR tubes or capillaries)

 Purified DNA samples (see “DNA purification”, page X)

 Spectrophotometer* and associated consumables (cuvettes, etc.), if required

 Nuclease-free water

 HRM-compatible PCR cycler* (see Table 5 in the Troubleshooting and FAQ section)

 Single- and/or multichannel pipets with nuclease-free tips*

 Nuclease-free microcentrifuge tubes

 Centrifuge* and rotor for PCR plate

*Instruments should be checked and calibrated according to the manufacturer’s recommendations.

Important Notes

General

Canon BioMedical recommends the following guidelines:

 Use sterile aerosol barrier pipet tips designed for PCR applications.

 Users should setup two physically separate workspaces; the first for PCR setup, and the second for PCR and post-PCR processing operations. This physical separation will minimize amplicon contamination that may impact data interpretation. Decontaminate the PCR workspace and labware (pipettes, tube racks, etc.) with UV light or with 10% bleach followed by wiping with isopropanol.

 Thaw frozen genomic DNA samples thoroughly at room temperature (15–25°C) or on ice before starting to prepare reactions.

DNA Extraction

There are no special limitations or assurances given for using DNA extracted with any commercial kits. The following kits and extraction methods have been used during testing by Canon BioMedical: Gentra Puregene Blood Kit for manual DNA extraction (QIAGEN), Genomic DNA Buffer Kit (QIAGEN), MagnaPure Compact automated system (Roche) with large and small Nucleic Acid Isolation kits, and QIACube automated system (QIAGEN) with QIAamp Blood DNA Mini Kit and QIAprep Spin Mini-Prep Kit.

HRMA is sensitive to changes in the salt and buffer components in samples. Therefore, Canon BioMedical strongly recommends that all samples (experimental and control) be prepared using the same extraction method to reduce variability in the subsequent analysis.

DNA Quality

All DNA samples should show consistent quality by UV spectrophotometric measurements or related methods. DNA purity after extraction may be determined at the same time the DNA concentration is measured.

 Concentration, as measured by A260, should be greater than or equal to 20 ng/µl. Samples with a concentration of less than 20 ng/ µl should be either excluded from analysis or concentrated by using ethanol precipitation. Samples with a higher concentration should be diluted to 20 ng/ µl by using nuclease-free water.

 The acceptable A260:A280 for use is 1.7 - 2.1.

Novallele Genotyping Assay Protocol

This protocol is for use with Novallele Genotyping Assays and Novallele Genotyping Mastermix together with a HRMA-compatible thermocycler. For the latest information on HRMA-compatible thermocyclers, please contact Canon BioMedical Support at 1-844-CANON-BIO.

Important points before starting

 Familiarize yourself with the HRMA-compatible thermocycler before starting this protocol. Refer to the thermocycler user manual, and follow the manufacturer's instructions for proper operation and maintenance.

 Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations.

 Make sure that no bubbles are introduced into the wells of the PCR plate during pipetting. If bubbles form, remove them by centrifuging the plate at 1000 rpm for 1 min (96-well plate), 2000 rpm for 2 min (384-well plate).This step is not necessary for all thermocyclers.

 Do not use DEPC-treated water. Use high-quality, nuclease-free water.

Procedure

1. Briefly invert or vortex all of the Novallele Genotyping Mastermix and the Novallele Genotyping Assays to mix the contents of the tube.

2. Briefly centrifuge the Novallele Genotyping Mastermix and the Novallele Genotyping Assays (10–15s) to bring the contents to the bottom of the tube.

Note: The Novallele Genotyping Mastermix is only active after heat activation; therefore reactions can be prepared and maintained at room temperature (15–25°C) for up to two (2) hours.

3. Prepare a reaction mix for each sample according to Table 1.

Recommendation: Prepare a reaction mix for a minimum of three (3) replicate reactions for each sample. Prepare ten percent (10%) more mix than is required to allow for imprecision in pipetting.

Recommendation: For each experiment, a No Template Control (NTC) and a positive homozygous wild type control are recommended. For the NTC and positive control sample, substitute nuclease-free water for the DNA in Table 1 to prepare the reaction mixture.

Table 1. Preparation of reaction mix

	Single test (1 test well per sample)	Technical replicates in triplicate (3 test wells per sample)
Novallele Genotyping Mastermix	3.6 µl	11 µl
Novallele Genotyping Assay	3.6 µl	11 µl
DNA	66.6 ng	220 ng
Nuclease-free Water	Up to final volume	Up to final volume
Final Volume	11 µl	33 µl

4. Add 10 µl of the reaction mixture to each well of either a 96-well plate or a 384-well plate using a single-channel pipet.

Note: Change pipet tips between each sample to avoid any cross-contamination between the wells.

5. Tightly seal the PCR plate as directed by the manufacturer.

6. Remove any air bubbles in any of the well plate by centrifuging the plate at 1000 rpm for 1 min (96-well plate); 2000 rpm for 2 min (384-well plate).This step is not necessary for QIAGEN Rotor-Gene.

7. Program the HRM-compatible thermocycler according to the PCR and HRM conditions provided on the Novallele Genotyping Assay Product Specification Sheet (which is also found on the Novallele Genotyping Assay's webpage at www.canon-biomedical.com).

8. Place the PCR plate in the thermocycler. Use a compression pad with the optical film-sealed plate/disc formats, if recommended by the manufacturer. Start the run.

9. After the run is finished, dispose of the PCR plate according to your institution/organization guidelines.

10. In order to analyze the data, please refer to the thermocycler manufacturer's instructions on analyzing HRM results. If you have questions, please contact Canon BioMedical Support at 1-844-CANON-BIO.

Note: Visually inspect the plate after the run for any signs of evaporation from any of the wells. If evaporation is observed, note which wells are affected, as this may affect the results of data analysis.

Note: Do not open any previously processed plate. Removing the strips or film releases PCR product into the air where it may contaminate and affect the results of future experiments.

Data Analysis

Canon BioMedical recommends that users analyze data using the instrument software. For additional recommendations, please contact Canon BioMedical Support at 1-844-CANON-BIO.

Appendix I

Using Novallele Reagents with User-designed Assays

Novallele Genotyping Mastermix is a laboratory-optimized fast PCR chemistry formulation that supports HRMA. Users with previously designed HRM assays may increase sample throughput and decrease reagent costs by converting to Novallele Genotyping Mastermix and 2× Novallele Oligo Dilution Buffer. The section below describes how a user should prepare their primers and optional unlabeled probes for use.

Important notes before starting

 The use of Novallele Genotyping Mastermix and 2× Novallele Oligo Dilution Buffer together is essential for a successful reaction. Do not replace the 2× Novallele Oligo Dilution Buffer with other commercial counterparts. The use of the Novallele Genotyping Assays and the Novallele Genotyping Mastermix is essential for a successful reaction.

 Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations.

 Use high-quality, nuclease-free water.

Generate Design-it-Yourself (DIY) Assay Mixtures

The use of a self-designed HRM assay with the Novallele Genotyping Mastermix and the 2× Novallele Oligo Dilution Buffer is a simple process that begins with creating an assay mixture that mimics the Novallele Genotyping Assay's chemistry. Users can create a working stock of their assays by diluting the primers/probes in the 2× Novallele Oligo Dilution Buffer. 2× Novallele Oligo Dilution Buffer contains all of the necessary chemistry for use DIY assays with the Novallele Genotyping Mastermix. The two tables below outline DIY Assay Mixtures for short amplicon HRM assays and for unlabeled probe HRM assays. If you have any questions regarding which table you should follow, please contact Canon BioMedical Support at 1-844-CANON-BIO.

Table 2. Preparation of a short amplicon DIY Assay Mixture*

	Concentration	Volume
2× Novallele Oligo Dilution Buffer	2×	300 µl
Forward Primer	100 µM	18 µl
Reverse Primer	100 µM	18 µl
Nuclease-free Water		264 µl
Final Volume		600 µl

*This mixture is the equivalent of a Novallele Genotyping Assay.

Note: Forward and Reverse Primers are designed and supplied by the user.

Table 3. Preparation of an unlabeled probe DIY Assay Mixture*

	Concentration	Volume
2× Novallele Oligo Dilution Buffer	2×	300 µl
Limited Primer	100 µM	3.6 µl
Excess Primer	100 µM	18 µl
Unlabeled Probe	100 µM	18 µl
Nuclease-free Water		260.4 µl
Final Volume		600 µl

*This mixture is the equivalent of a Novallele Genotyping Assay

Note: Limited, Excess Primers, and Unlabeled Probes are designed and supplied by the user.

Note: Table 3. describes a 1:5 ratio of limited to excess primer, users may need to adjust this ratio based on assay design. For guidance, please contact Canon BioMedical Support at 1-844-CANON-BIO.

Using DIY Assay Mixtures in genotyping experiments

After preparing a DIY Assay mixture, users should follow the Novallele Genotyping Assay Protocol as described above starting on pg. #, substituting the DIY Assay mixture for the Novallele Genotyping Assay in the protocol. While the conversion of a DIY Assay for use with the Novallele Genotyping Mastermix is a simple procedure, the user may need to optimize cycling parameters to obtain optimal results. For guidance, please contact Canon BioMedical support at 1-844-CANON-BIO.

Table 4. Preparation of reaction mix

	Single test (1 test well per sample)	Technical replicates in triplicate (3 test wells per sample)
Novallele Genotyping Mastermix	3.6 µl	11 µl
DIY Assay mixture	3.6 µl	11 µl
DNA	66.6 ng	220 ng
Nuclease-free Water	Up to Final volume	Up to Final volume
Final Volume	11 µl	33 µl

Troubleshooting and FAQs

This troubleshooting and FAQs section supports users by answering Frequently Asked Questions and providing helpful hints from the scientists that developed the Novallele Products. For updated information, see also the Frequently Asked Questions page at www.canon-biomedical.com. If this section does not address your questions or concerns, please contact Canon BioMedical Support at 1-844-CANON-BIO.

On what types of instruments can these be run?

Canon BioMedical has established performance of the Novallele Genotyping Assays, Novallale Genotyping Mastermix and 2× Novallele Oligo Dilution buffer on the following instrument platform: BioRad CFX.

Other HRMA platforms such as Roche LC480, ThermoFisher QuantStudio, Rotor-GeneQ, Roche LightCycler® 2.0 and Biofire LS32 may be compatible instruments, however testing has not been performed by Canon BioMedical. For other platforms listed above, verification or validation of suitability is recommended, please contact Canon BioMedical Support at 1-844-CANON-BIO for additional questions or concerns.

What types of source DNA can be used for the Novallele assays?

Canon BioMedical has tested genomic DNA extracted from cell lines and blood as well as synthetic samples including plasmids and double-stranded linear segments.

What DNA extraction methods are suitable for the Novallele assays?

Canon BioMedical has tested DNA extracted by many methods with comparable results. Any method that yields suitable concentration, purity, and integrity is acceptable for use, but it is essential that all samples be prepared using the same method to generate optimal genotyping results.

How many samples can be tested with a single vial of the Novallele Genotyping Assay?

Canon BioMedical recommends using 3.6µL of Novallele Genotyping Assay per reaction and thus sufficient mix is provided for approximately 166 assays (600 µL/3.6 µL=166) per single vial. Please be advised that pipetting errors may reduce the number of possible assays.

What control(s) should I use with my Novallele Genotyping Assay?

Canon BioMedical routinely uses both negative and positive controls for all of our assays. Canon BioMedical recommends using a no template control (negative) to rule out any environmental contamination and a homozygous wild-type control (positive) for each run. To the extent possible, the inclusion of additional controls such as heterozygous or homozygous variants is recommended, but not required.

Where can I find the recommended reaction conditions?

The applicable product specification sheet contains the detailed assay conditions for each Novallele Genotyping Assay. The specification sheets are included with the Novallele Genotyping Assay, as well as available at www.canon-biomedical.com.

Is there a recommended analysis method for the assays?

Please refer to the user manual of your PCR instrument for the analysis software. Generally both small amplicon and probe-based assays are analyzed using the first derivative of the melting curve, known as the derivative plot.

There is an additional bump/hump/shoulder on one of my samples, what does this mean?

Extra features on the melt curve can originate from many sources. Additional melt curve features may simply be a consequence of the magnitude of smoothing used for data analysis, but could also be an indicator of an additional sequence variation within the region being analyzed or contamination.

Another possibility is that the additional melt feature is an artifact caused by an instrument error or particulate contamination that disrupted the imaging process. Alternatively, the DNA quality may be compromised thus Canon BioMedical recommends analyzing the sample DNA to confirm an A260/A280 ratio within the range of 1.7-2.1.

Canon BioMedical recommends repeating the assay on this DNA sample if possible.

References

Canon BioMedical maintains a database of scientific publications utilizing Canon BioMedical products. For a list of references, please contact Canon BioMedical Support at 1-844-CANON-BIO.

Ordering Information

Product	Catalog Number	Description
Novallele Genotyping Mastermix	40018	Rapid PCR chemistry formulation for High Resolution Melting Analysis
Novallele Genotyping Assays	Assay dependent	Genetic variation-specific PCR assay
2× Novallele Oligo Dilution Buffer	40017	Primer/Probe dilution buffer for use with Novallele Genotyping Mastermix

Notes

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