Qexp-MDx APOE Genotyping Kit

Direct multiplex PCR testing for the genotyping of human APOE

Operation Manual

Ver. EN-D

Research Use Only

REF C900400-24 & C900400-48
C903402-24 & C903402-48
C903401-24 & C903401-48

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Intended Use

Apolipoprotein E (APOE) is a 34 kDa protein involved in fat metabolism. It is located on human chromosome 19 and is associated with Alzheimer's disease and cardiovascular disease. *APOE* gene has three polymorphic alleles (ε2, ε3, and ε4) that encode three protein isoforms—E2 (Cys112/Cys158), E3 (Cys112/Arg158), and E4 (Arg112/Arg158), everyone inherits one of three alleles from each parent and resulting in six possible genotypes—ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4. According to previous studies, if there is an ε4 in *APOE* may cause a high risk of late-onset Alzheimer's disease. Furthermore, Familial type III hyperlipoproteinemia is frequently mentioned as associated with the *APOE* ε2/ε2.

The *Qexp*-MDx APOE Genotyping Kit is intended for molecular biology application testing. This product is not intended for the diagnosis, prevention, or treatment of a disease. Results are for the identification of the human apolipoprotein E (*APOE*) genome type in oral specimens or blood. Testing with the *Qexp*-MDx APOE Genotyping Kit is intended for use by trained laboratory personnel in molecular biology techniques.

Product Information

Qexp-MDx APOE Genotyping Kit for Blood

Product Description

The *Qexp*-MDx APOE Genotyping Kit for Blood (cat# C900400-24, C900400-48) includes the assay and positive control for direct multiplex PCR testing for identifying the genome type of *APOE* in human blood specimens. The *Qexp*-MDx APOE Genotyping Kit for Blood includes the following components:

- **Direct Lysis assay**
  Sample lysis assay contains Proteinase K and DNA Lysis Buffer.
- **Direct APOE Kit assay**
  Direct multiplex PCR assay – multiplexed assays contain a primer set specific to the SNP region of the *APOE* in the human genome.
- **Positive Control-APOE**
  The positive control contains the DNA of *APOE* which are targets for each primer set and is used to test whether the assay is performing correctly. The PCR product of the positive control will be set as size marker.

Contents and Storage

Table 1 Direct APOE Kit for Blood, 24 and 48 reactions

Label	Component	Amount (μL)		Storage
		24 rxns	48 rxns	
Direct Lysis assay				
PK	Proteinase K	132	264	-20℃
L1	DNA Lysis Buffer	1008	2016	-20℃
APOE multiplex PCR assay				
1	Primer mix-APOE	60	120	-20℃
A1	Direct PCR PreMix	300	600	-20℃
B1	Enhanced Buffer	120	240	-20℃
PC	Positive Control-APOE	60	120	-20℃
W	Nuclease-Free Water	150	300	-20℃

- All components should be stored at -20℃ immediately after unpacking.
- It is recommended to aliquot the reagents to avoid the risk of efficiency decrease by being thawed and frozen more than 2 times.

Qexp-MDx APOE Genotyping Kit for Oral Swab

Product Description

The *Qexp*-MDx APOE Genotyping Kit for Oral Swab (cat# C903402-24, C903402-48) includes the assay and positive control for a direct multiplex PCR testing for identifying the genome type of *APOE* in human oral swab specimens. Alternatively, the *Qexp*-MDx APOE Genotyping Kit (cat# C903401-24, C903401-48) is the reagent-only version kit, not including the sample collection tool. The *Qexp*-MDx APOE Genotyping Kit for Oral Swab includes the following components:

- **Specimen Collection**
  Flocked, foam, or nylon swab with plastic shafts and the transport tube with UTM (cat# C9000101-024, C9000101-048).
- **Direct Lysis assay**
  Sample lysis assay contains Proteinase K and DNA Lysis Buffer.
- **Direct APOE Kit assay**
  Direct multiplex PCR assay – multiplexed assays contain a primer set specific to the SNP region of the *APOE* in the human genome.
- **Positive Control-APOE**
  The positive control contains the DNA of *APOE* which are targets for each primer set and is used to test whether the assay is performing correctly. The PCR product of the positive control will be set as size marker.

Contents and Storage

Table 2 Direct APOE Kit for Oral Swab, 24 and 48 reactions

Label	Component	Amount (µL)		Storage
		24 rxns	48 rxns	
Specimen Collection				
	Oral Swab with Collection Tube contains 3mL UTM[a]	24	48	RT
Direct Lysis assay				
PK	Proteinase K	62.4	124.8	-20℃
L1	DNA Lysis Buffer	480	960	-20℃
APOE multiplex PCR assay				
1	Primer mix-APOE	60	120	-20℃
A1	Direct PCR PreMix	300	600	-20℃
B1	Enhanced Buffer	120	240	-20℃
PC	Positive Control-APOE	60	120	-20℃
W	Nuclease-Free Water	150	300	-20℃

[a]Oral Swab with Collection Tube contains 3mL UTM (C9060001-024, C9060001-048) should be purchased separately.

- All components should be stored at -20℃ immediately after unpacking, except the

Oral Swabs with UTM which are stored at room temperature (RT).

- It is recommended to aliquot the reagents to avoid the risk of efficiency decrease by being thawed and frozen more than 2 times.

Required materials not supplied in kit

Table 3 Direct sample collection tool (Not Provided)

Item	Source
Direct collection tool	
EDTA blood collection tubes	any supplier*

Table 4 Required Materials (Not Provided)

Item	Source
Consumables	
Disposable powder-free gloves and lab coats	any supplier*
Disposable pipette tips with the aerosol barrier	any supplier*
10% bleach or other appropriate cleaning solution that degrades nucleic acids	any supplier*
75% ethanol for bench cleaning	any supplier*
PCR tubes, plates, or strip tubes for the thermocycler being used	any supplier*
1.5 mL microcentrifuge tubes, nuclease-free	any supplier*
Equipment	
Several micropipettes capable of pipetting volumes from 2μL to 1000μL	any supplier*
Vortex and centrifuge	any supplier*
PCR workstation, for master mix preparation and setup	any supplier*
Qamp$_{mini}$ Thermal cycler	BiOptic Cat. C310200
Qsep$_{400}$™ Bio-Fragment Analyzers *Qsep*$_{100}$™ Bio-Fragment Analyzer *Qsep*$_{1}$™ Bio-Fragment Analyzer *Qsep*$_{1}$-Lite™ Bio-Fragment Analyzer	BiOptic Cat. C400100 BiOptic Cat. C100100 BiOptic Cat. C100001 BiOptic Cat. C100001-L
Qairbox	BiOptic Cat. C104301-00
Qsep series cartridge kits-S2 standard cartridge	BiOptic Cat. C105201

*The required materials not provided in this kit can be ordered from another major laboratory supplier.

Assay limitations

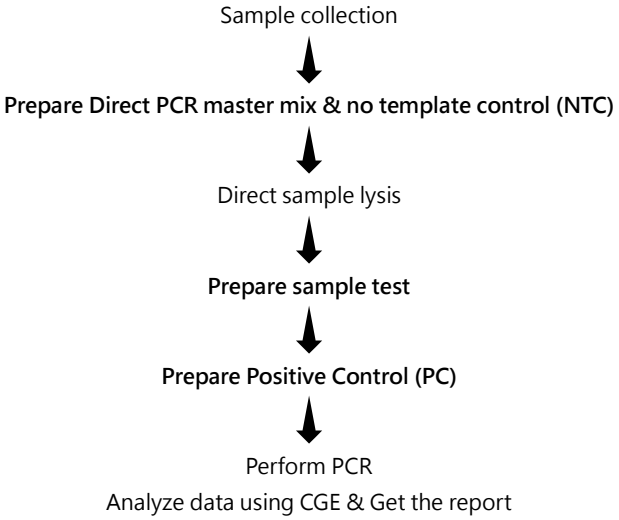
- Specimen types other than oral specimens and blood samples from the EDTA blood collection tube could not be worked with this assay.
- Having milk or food full of calcium before sample collecting of the oral swab will cause the PCR process failure.
- The oral specimens with sputum may not be worked with this assay.
- The collection, transportation, and storage of the samples must follow the appropriate procedures and conditions. Inappropriate uses may cause a decrease in the efficiency and sensitivity of the assay to detect the genome sequence.
- Failed results may be caused by:
  - Improper sample collection or extraction
  - Degradation of the genomic DNA in the procedure
  - The presence of the PCR inhibitors
- Positive no template control (NTC) results may be caused by:
  - Cross-contamination during sample collection or preparation
  - Genome contamination between samples
  - Cross-contamination by PCR amplicon
  - Contamination by improper preparation of positive control

Warning and precautions

To avoid contamination results, the Direct APOE kit workflow should use different areas or rooms. A dedicated PCR workstation for the preparation of the direct PCR assay would be ideal, and the analysis of the PCR product should be carried out in a separate area. The tube with positive control should be handled with great care to avoid contamination problems.

- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Always use pipette tips with aerosol barriers. Tips should be sterile and DNases- and RNases-free.
- Reagents must be stored and handled as specified in the content and storage section of the kits (Table 1, 2).
- To prevent contamination results, be sure to use different pipettes for the preparation of direct PCR reagents and master mixes, for the preparation of Sample Lysates, and for pipetting the positive control.
- Modifications of assay reagents or assay protocols are not permitted.
- Do not use the kit after the expiration date.
- Dispose of waste in compliance with local, state, and federal regulations.

Workflow



※ IMPORTANT

- To avoid contamination results, always use different areas and pipettes for preparing reagents, adding positive control, adding sample lysate, and analyzing PCR amplicon.
- Always use tips with the aerosol barrier to avoid contamination.
- For preparing the reactions every time, include the following controls:
  One positive control
  One no template control (NTC)

The Direct PCR PreMix can be stored at 4℃ for a week. It is recommended to prepare an appropriate amount of the above reagents, then do the Direct APOE test immediately. The Direct PCR master mix could be prepared fresh at room temperature before use.

The genomic DNA from oral or blood specimens could be released by using the required DNA Lysis Buffer, then be amplified by the Direct PCR assay. In the process, the primers anneal to the SNP-specific regions of the human *APOE* gene.

The PCR product is analyzed by the *Qsep* series Bio-Fragment Analyzers, then provides the report automatically by the software *Q-Analyzer* (not required in the kit).

Guidelines for Sample Collection for Blood

IMPORTANT

- For blood sample collection, please follow the guidelines announced by the local governments for appropriate operation.
- Only performed by well-trained medical personnel.
- Only specimens collected by EDTA Blood Collection Tube (lavender/purple cap) are available for this kit.
- All specimens should be regarded as potentially infectious and standard precautions guidelines should be followed during sample collection and handling.

Guidelines for Sample Collection for Oral Swab

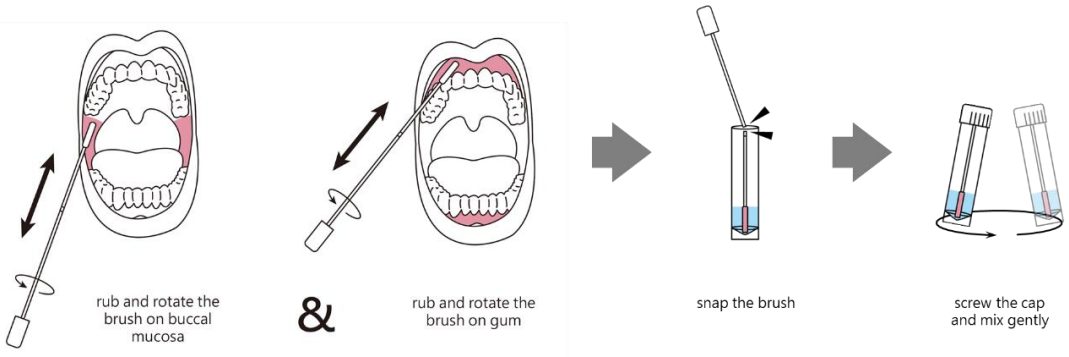
IMPORTANT

- Avoid drinking milk or having food full of calcium before oral swab collection.
- Before sample collection, rinse the mouth with clean water for 10 seconds to remove microorganisms, and remnants of food and drink.
- Ensure that there was no eating, drinking, smoking, chewing gum, brushing teeth, or use of mouthwash after rinsing the mouth.

Oral specimen collection from subject

1. Open the Specimen Collection Brush (Swab).
2. Rub and rotate the brush on the buccal mucosa and gum 10 times on each side to collect cells.
3. Insert the brush into the supplied Collection Tube with UTM.
4. Snap the brush in the tube, then screw the cap of the Collection Tube.
5. Mix well gently to release the cells on the brush into UTM.

※ Can be stored at -20℃ for a week.



Preparation of Direct PCR Reaction

Prepare Direct PCR master mix

1. Thaw the kit at room temperature.
2. Gently vortex the Primer mix-APOE and Direct PCR PreMix.
3. Centrifuge briefly to collect the liquid at the bottom of the tube.
4. Prepare the Direct PCR master mix in the PCR tube or 1.5 mL microcentrifuge tube:

Table 5 Preparation of Direct PCR master mix

Component	Volume per sample	Volume for n samples plus 2 controls	Volume for 46 samples plus 2 controls
Primer mix-APOE	2.5	2.5 x (n+2)	120
Direct PCR PreMix	12.5	12.5 x (n+2)	600
B1 Enhanced buffer	5	5 x (n+2)	240
TOTAL (µL)	20	20 x (n +2)	960

5. Mix by pipetting or gently inverting the Direct PCR master mix, then centrifuge briefly to collect the liquid at the bottom of the tube.
6. Transfer every 20 µL Direct PCR master mix to a PCR tube.

Prepare blood sample lysate

1. Prepare Lysis Solution by mixing 5.5 µL Proteinase K and 42 µL DNA Lysis Buffer together for a blood sample.
2. Mix well the EDTA Blood Collection Tube by inverting gently before use.
3. Transfer 2.5 µL blood sample in a clean PCR tube.
4. Add 47.5 µL Lysis Solution.
5. Mix well by pipetting or gently vortex.
6. Incubate at 95℃ for 5 mins to inactivate Proteinase K, then cool down to 4℃.

Prepare oral specimen lysate

1. Mix well gently by inverting the UTM Collection Tube before use.
2. Take 20 µL mixture of sample and UTM in a clean PCR tube.
3. Add 2.6 µL Proteinase K, and incubate at room temperature for 1 min.
4. Incubate at 95℃ for 5 mins to inactivate Proteinase K, then cool down to 4℃.
5. Add 20 µL DNA Lysis Buffer (L1) and mix well by gently vortex or pipetting.
6. Incubate at room temperature for 10 mins.

Prepare Direct PCR reaction

Table 6 The preparation list of all PCR reaction

Component	Volume per reaction		
	NTC	Sample reaction	Positive control
Direct PCR master mix	20	20	20
specimen lysate	-	5	-
Nuclease-Free Water	5	-	-
Positive Control-APOE	-	-	5
TOTAL (μL)	25	25	25

※ Do not use the same pipette to add water, specimen lysates, and positive control. Do not add positive control in the same area as reagents preparing. Avoid producing bubbles in the PCR tube.

※ Always prepare the NTC first, and the positive control last to avoid cross-contamination.

1. For NTC, transfer 5 μL Nuclease-Free Water to a prepared Direct PCR master mix.
2. For sample reaction, transfer 5 μL of the oral specimen, or blood lysate to a prepared Direct PCR master mix.
3. For positive control, transfer 5 μL Positive Control-APOE to a prepared Direct PCR master mix after thawing it on ice.
4. Mix by inverting briefly or vortex gently after addition, then centrifuge briefly to collect the liquid at the bottom.

Perform Direct PCR using the Thermal Cycler

Before using the thermal cyclers, please read the operation manual which the manufacturer provided and pay attention to the safety information. To guarantee problem-free operation, please follow the instructions and safety precautions to ensure safe operation.

Setup and Run

1. Confirm the thermal protocol as below:

Table 7 The PCR program of the Direct APOE assay

Step	Number of cycles	Temperature (℃)	Time
Activation	1	95	10 minutes
Denaturation	35	95	30 seconds
Anneal		67	30 seconds
Extension		72	30 seconds
Extension	1	72	5 minutes
	1	4	hold

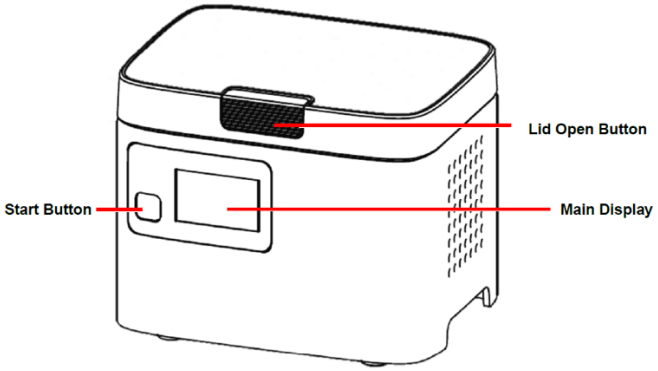
2. After the PCR is complete, transfer the PCR tube to the CGE system for analysis.

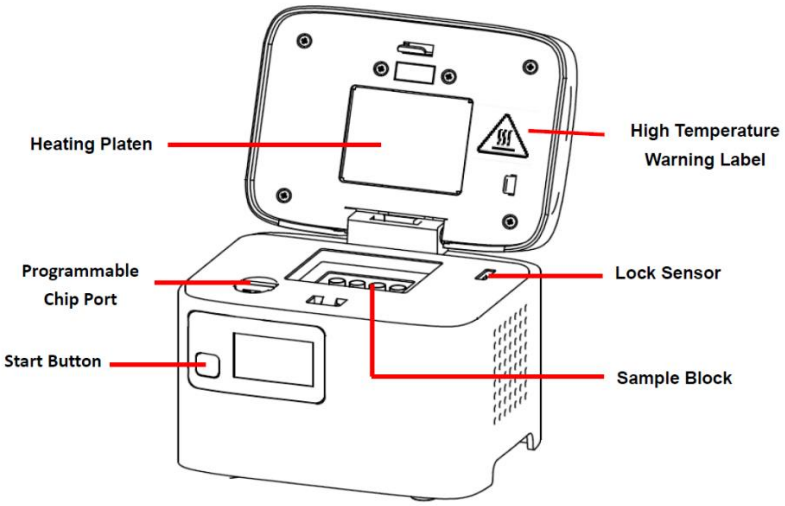
Perform PCR using *Qamp*$_{mini}$™ Thermal Cycler

General Description

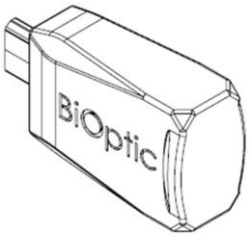
Before using the *Qamp*$_{mini}$™, please read this operation manual carefully and pay attention to the safety information. To guarantee problem-free operation, please follow the instructions and safety precautions to ensure the safe operation of the *Qamp*$_{mini}$™.

Qamp$_{mini}$™ is a portable PCR thermocycler. It contains a centrally positioned Peltier heating & cooling module for 1-8 samples. This design leads to accuracy in analysis and cost efficiency without sacrificing performance and quality. With the compact size and one start button to operate design, *Qamp*$_{mini}$™ is the ideal instrument for laboratories or classrooms and in the fields of epidemiology, veterinary, food testing, pathogen detection, ecology, archaeology research, and others.





Programmable Chip is for saving PCR programs according to applications. The program details can be edited by the ***Qamp***$_{mini}$™ writer and will be recognized automatically when the Programmable Chip is inserted into the port.



For more detailed information on the instruments, please find them on our website.

Setup and Run

1. Confirm or edit the thermal protocol following Table 7 by the ***Qamp***$_{mini}$™ writer.
2. Insert Programmable Chip into the port, then turn on the power switch.
3. Open the lid and load the reaction vessel. (only 0.2 mL tubes are available)
4. Close the lid and wait until the lid is heated to 60℃, the machine will make two beeps.
5. Press the start button to proceed with the program.
6. After the PCR is complete, press the start button for 3 seconds to stop the program. Then transfer the PCR tube to the CGE system for further analysis.

Analysis and Results

For better sensitivity and resolution, please use capillary gel electrophoresis (CGE) systems, such as BiOptic *Qsep* series Bio-Fragment Analyzers, to analyze the Direct PCR amplicon. It is available to perform with any brand's CGE system. Before using the capillary gel electrophoresis system, please read the operation manual which the manufacturer provided and pay attention to the safety information.

Instrument and Software

Table 8 Instrument and software compatibility

Instrument	*Q-Analyzer*™
$Qsep_{400}$™ Bio-Fragment Analyzer	Version 4.1.0 or later
$Qsep_{100}$™ Bio-Fragment Analyzer	
$Qsep_{1}$™ Bio-Fragment Analyzer	
$Qsep_{1}$-Lite™ Bio-Fragment Analyzer	Version 3.1.0 or later

Table 9 Software Requirements

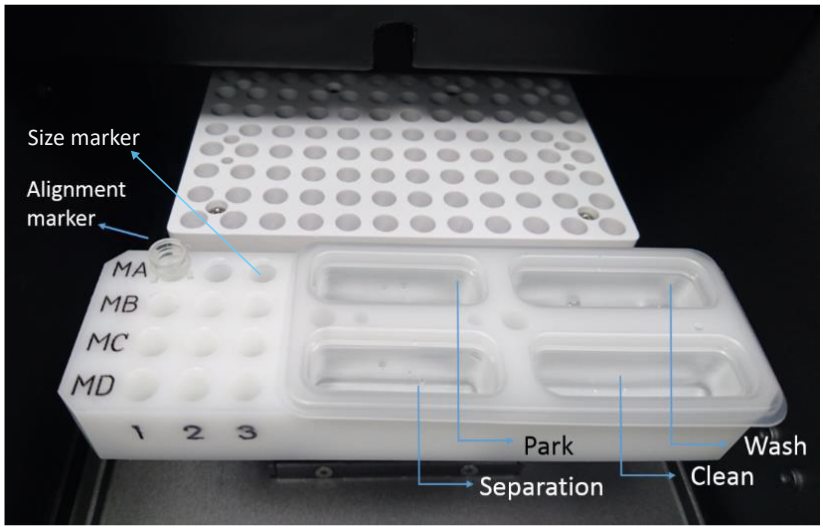
	Minimum	Recommended
OS	Windows 10 32 bit	Windows 10 64 bit
CPU	Intel Core i5	Intel Core i7
RAM	8G RAM	16G RAM
HDD	500 GB	1 TB
PORT	RJ45 x 1 USB 3.0 x 1	RJ45 x 2 USB 3.0 x 2
Screen Resolution	1280 x 800	1920 x 1080

Perform Assay using *Qsep*$_{100}$™ Bio-Fragment Analyzer

Setup and Run

1. **"Connect"** *Qsep*$_{100}$.

   "Change Buffer" to place Buffer Tray (filled up with water and Separation Buffer in the right position), Alignment Marker 20 bp & 1000 bp (C109100) at MA1, PCR product of positive control as Size Marker (C900400) at MA3.

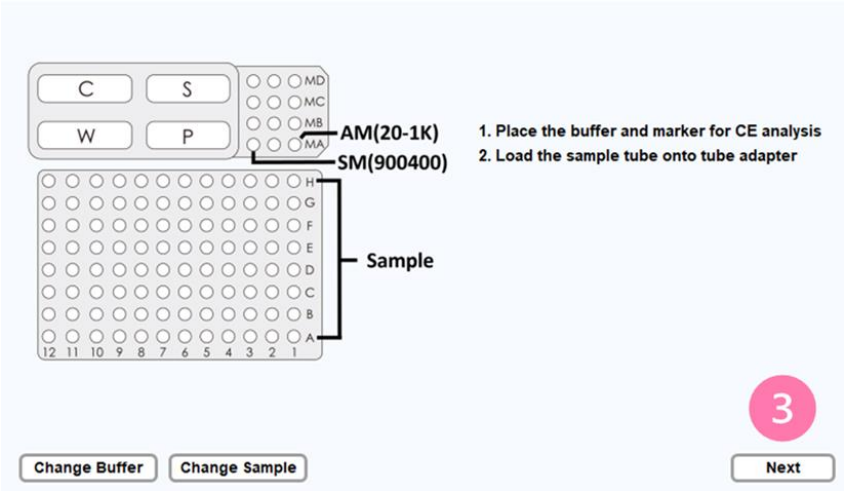


   "Change Sample" to place the samples. Remember to remove the cap of PCR tube. (For more information, please refer to *Qsep* Series Operation Manual)

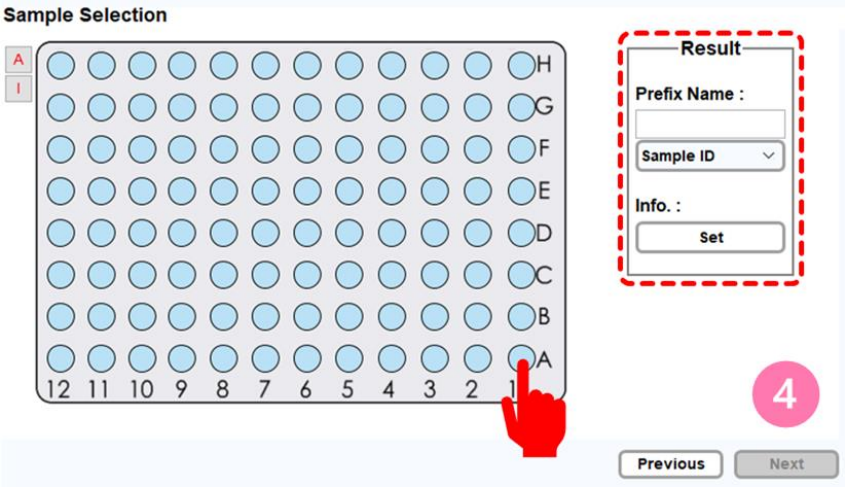
2. **"Latch"** the S2 cartridge. (For more information, please refer to the F0061-S2_Cartridge User Guide)

3. Switch to **"Assay"** and select the icon in the following order: **"MDx"** → **"Genetic Disorder"** → **"APOE"** .

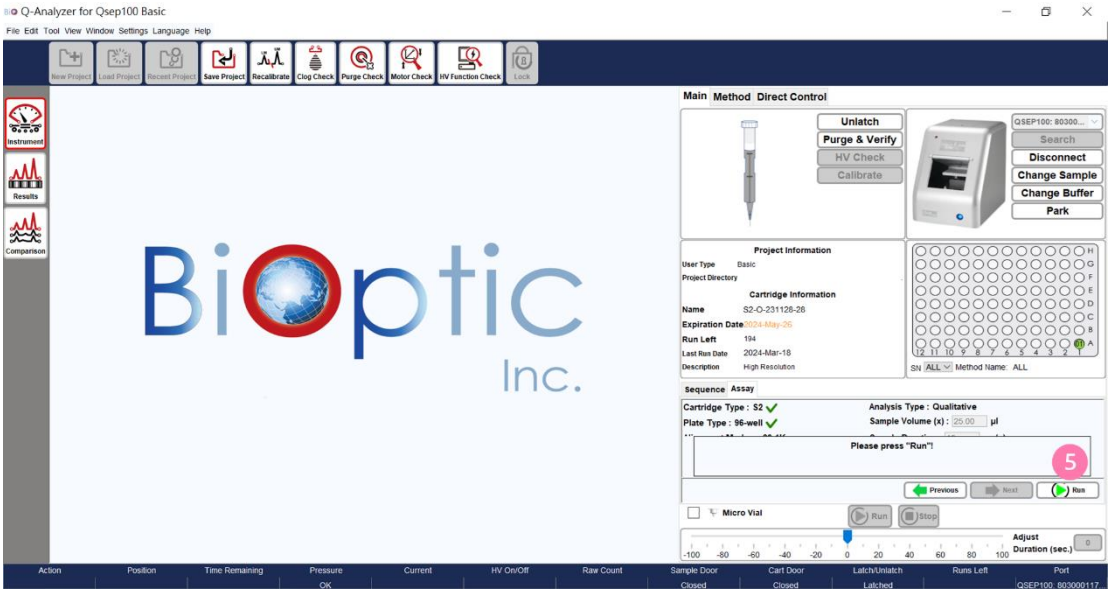
4. Click **"Next"** to select the sample position.



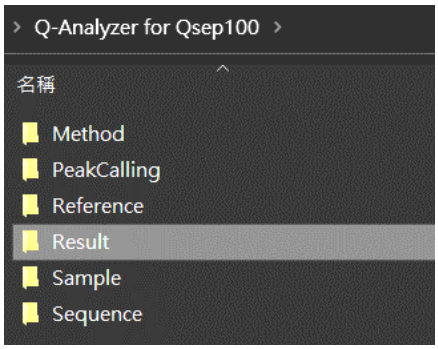
5. Select the sample position and click **"Next"** .
6. (Optional) Type the result name in the field of **"Prefix Name"** and click **"set"** to type the desired information of the sample.



7. Click "**Run**" to start the analysis.



8. The PDF report can be found in the "**Result**" folder with the date of the test.



The results of quality control (NTC and Positive Control)

- Ensure that there is no signal of the target sequences revealed in the NTC.
  - ➢ If the NTC has any signal, the reagents might be contaminated in the procedure.
- Ensure that all signals of the target sequences are shown in the positive control.
  - ➢ If the positive control did not show any signal, it may cause by the degradation of the reagents. Please contact the distributor in your area.

The result of Direct APOE assay

The data shown below were run by BiOpitc's Capillary Gel Electrophoresis system, $Qsep_{100}$ fragment analyzer with S2 cartridge.

Table 10 Amplicon of *APOE* polymorphisms

There are six different results, including three homozygote and three heterozygote genotypes as the table below. The cysteine and arginine interchanges at codons 112 and 158, leads to the polymorphism of the *APOE* gene. Therefore, we can amplify different size amplicons to identify the genotype of *APOE*.

Genotype		Target Sequence (bp)				
		1st, codon 112 (rs429358)[a]		2nd, codon 158 (rs7412)[a]		Common band
		TGC[b]	CGC[b]	TGC[b]	CGC[b]	
		Cys[c]	Arg[c]	Cys[c]	Arg[c]	
Homozygote	ε2/ε2	112	--	253	--	514
	ε3/ε3	112	--	--	308	
	ε4/ε4	--	444	--	308	
Heterozygote	ε2/ε3	112	--	253	308	
	ε2/ε4	112	444	253	308	
	ε3/ε4	112	444	--	308	

[a] SNP (Single Nucleotide Polymorphism) ID

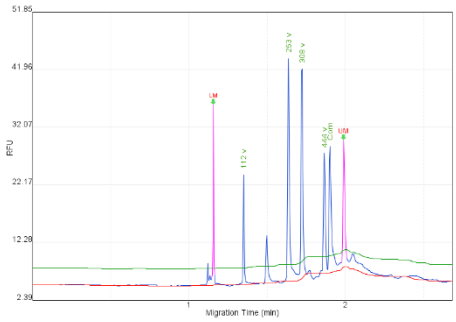
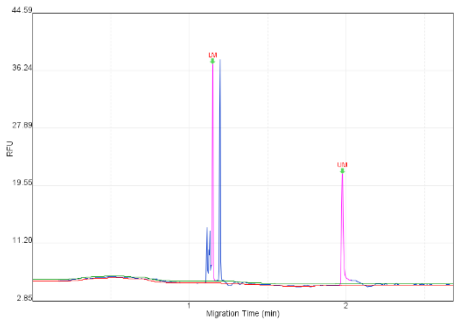
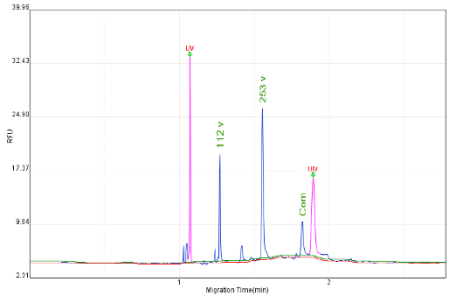
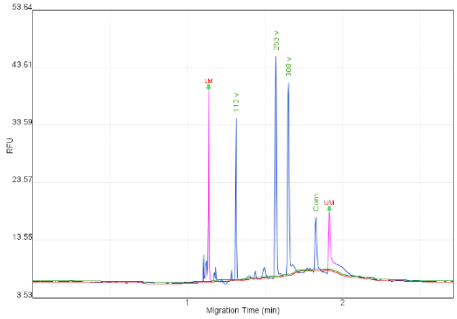
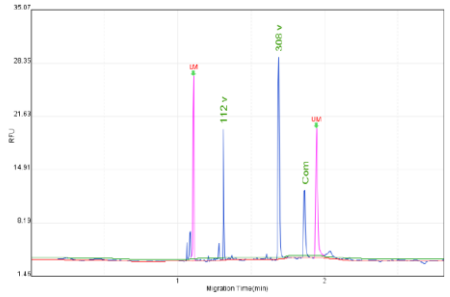
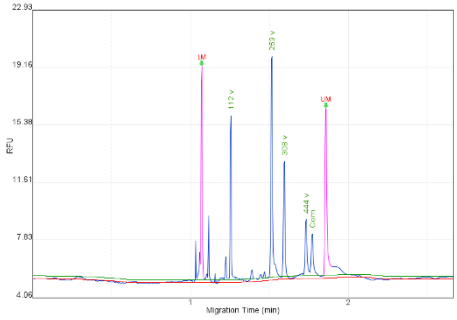
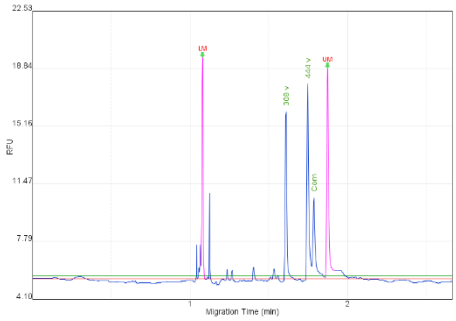
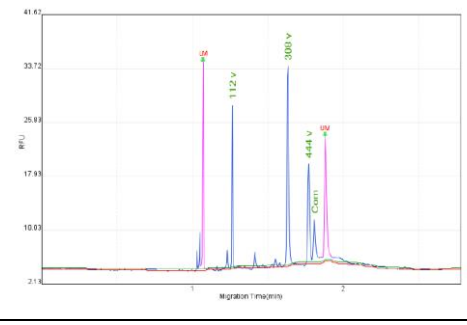
[b] Nucleotide

[c] Amino acid

-- means this target will not be amplified

Table 11 The CGE results of Direct APOE assay

DNA amount in the sample may cause variation in the signal level. There is an amplicon around 183bp that might be amplified during the process, and it won't affect the test result.

Type and Result data	
Positive Control	**NTC**
	
ε2/ε2: 112, 253 bp	**ε2/ε3**: 112, 253, 308 bp
	
ε3/ε3: 112, 308 bp	**ε2/ε4**: 112, 253, 308, 444 bp
	
ε4/ε4: 308, 444 bp	**ε3/ε4**: 112, 308, 444 bp
	

Com: common band

Documentation and Support

Related documentation

For other related documents of the PCR kits, *Qsep* series instruments, BiOptic Catalog, etc., please visit the Download page on BiOptic's website.

Customer and technical support

For more information about the detection kit or instruments, please visit BiOptic's website or contact the Distributors in your area.

Symbol Explanation

Table 12 Explanation of the symbols used in the labeling

Symbol	Symbol Title	Explanatory Text
	Manufacturer	Indicates the medical device manufacturer
	Catalog number	Indicate the manufacturer's catalog number to identify the medical device