Qexp-MDx ADRB3 Genotyping Kit

Direct multiplex PCR testing for the genotyping
of human ADRB3

Operation Manual

Ver. EN-B

Research Use Only

REF C903601-024 & C903601-048

BiOptic Inc.
(23141) 4F., No.108-4, Minquan Rd., Xindian Dist.,
New Taipei City, Taiwan (R.O.C.)

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Intended Use

What is the obesity gene?

Why do we often hear people say that "Even drinking water will make me fat"? Some people eat and drink all day long but still maintain their shape, however some people diet and exercise desperately but can't get rid of the rising weight. Did you know that in addition to acquired effects, obesity is also related to innate genes? Obesity genes are a general term for a series of genes related to obesity. Common obesity genes currently include ADRB2, ADRB3, UCP1, PPARγ, GNB3, and Sema3. This test is for typing ADRB2, ADRB3, and UCP1, three genes related to fat metabolism, to analyze the risk of obesity.

Adrenergic receptor β3 (ADRB3)

ADRB3 is mainly distributed on the cell membrane of adipocytes and its function is similar to *ADRB2*. The difference is that ADRB3 participates in intercellular signaling and is responsible for fat dissolution and thermogenic reactions. These reactions are regulated by the autonomic nervous system. The gene mutation point of ADRB3 is at the 190th nucleotide. When the nucleotide mutates from T to C, it will lead to easy accumulation of fat and increase the risk of upper body obesity. Research reports also indicate that people with C gene mutations have lower autonomic nervous activity than normal people. At the same time, it was also found that their basal metabolic rate was also low, making it difficult for them to undergo fat dissolution and thermogenic reactions.

The *Qexp*-MDx ADRB3 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 identifying the genome type of the ADRB3 in oral specimens. Testing with the *Qexp*-MDx ADRB3 Genotyping Kit is intended for use by trained laboratory personnel in molecular biology techniques.

Product Information

Qexp-MDx ADRB3 Genotyping Kit

Product Description

The *Qexp*-MDx ADRB3 Genotyping Kit includes the assay, positive control for a direct multiplex PCR testing for identifying the genome type of *ADRB3* in human oral specimens. The *Qexp*-MDx ADRB3 Genotyping Kit includes the following components:

- **Direct Lysis assay**
  Sample lysis assay contains Proteinase K and DNA Lysis Buffer.
- **Direct ADRB3 Kit assay**
  Direct multiplex PCR assay – multiplexed assays contain *ADRB3* primer set specific to the SNP region of the *ADRB3* in the human genome.
- **Positive Control-ADRB3**
  Positive control contains the DNA of *ADRB3* which are targets for each primer set and is used to test whether the assay is performing correctly.

Contents and Storage

Table 1 Direct ADRB3 Kit 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℃
ADRB3 multiplex PCR Assay				
1	Primer mix-ADRB3	48	96	-20℃
A1	Direct PCR PreMix	300	600	-20℃
PC	Positive Control-ADRB3	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 2 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 preparing 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

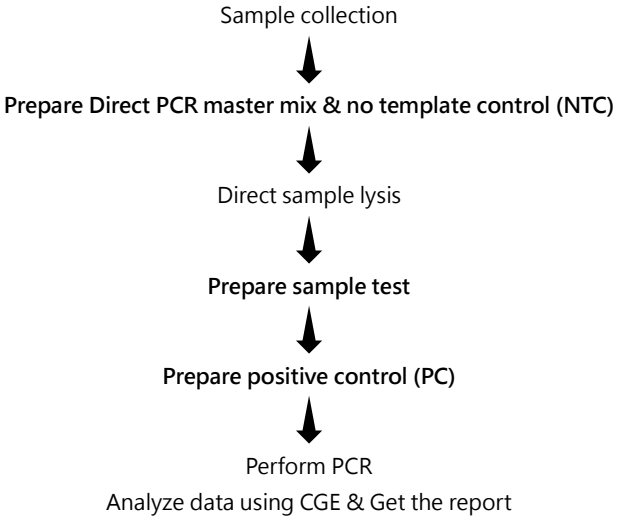
- This kit is performed by using oral specimens.
- Specimen types other than those mentioned above could not be worked with this assay.
- Milk or food rich in calcium will cause the PCR process failure.
- The oral specimens with sputum may not be worked with this assay.
- The collection, transportation, 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 ADRB3 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 Table 1.
- 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.
- Keep samples and reagents on the ice or freezing container during use.
- For preparing the reactions every time, include the following controls:
  One positive control
  One no template control (NTC)

The A1 PCR PreMix can be stored at 4℃ for a week. It is recommended to prepare an appropriate amount of above reagents, then do the Direct *ADRB3* test immediately. The reaction master mix could be prepared fresh in room temperature before use.

The genomic DNA from oral 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 human *ADRB3* gene.

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

Guidelines for Sample Collection for Oral Swab

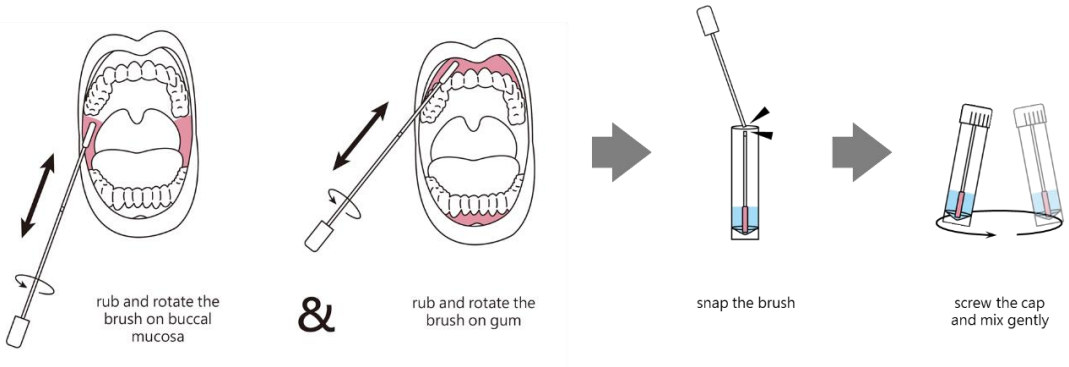
IMPORTANT

- Avoid drinking milk or having food rich in 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 is no eating, drinking, smoking, chewing gum, brushing teeth, or use of mouthwash after rinsing the mouth.

Oral specimen collection from the subject

1. Open the Specimen Collection Brush (Swab).
2. Force the brush a little bit on your buccal mucosa, then 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.



Prepare Direct PCR Master Mix

1. Thaw the kit at room temperature.
2. Gently vortex the Primer mix-ADRB3 and Direct PCR PreMix.
3. Centrifuge briefly to collect the liquid at the bottom of the tube.
4. Prepare the desired Direct PCR master mix in the PCR tube or 1.5 mL microcentrifuge tube.

Table 3 Preparation of Direct PCR master mix

Component	Volume per sample	Volume for n samples plus 2 controls	Volume for 22 samples plus 2 controls
Primer mix-ADRB3	2	2 x (n+2)	48
Direct PCR PreMix	12.5	12.5 x (n+2)	300
Nuclease-Free Water	5.5	5.5 x (n+2)	132
TOTAL (µL)	20	20 x (n +2)	480

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 Direct PCR reaction

Table 4 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-ADRB3	-	-	5
TOTAL (µL)	25	25	25

※ Do not use the same pipette to add water, oral specimen lysates, and positive control. Do not add positive control in the same area as reagent preparation. Avoid producing bobbles in the PCR tube.

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

1. For NTC, Transfer 5 µL Nuclease-Free Water in the prepared master mix.
2. For sample reaction, Transfer 5 µL of the oral specimen lysate to a prepared Direct PCR master mix.
3. For positive control, Transfer 5 µL Positive Control-ADRB3 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 that 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 5 The PCR program of the Direct ADRB3 assay

Step	Number of cycles	Temperature (℃)	Time
Activation	1	95	10 minutes
Denaturation	35	95	10 seconds
Anneal		62	10 seconds
Extension		72	20 seconds
Extension	1	72	5 minutes
	1	4	hold

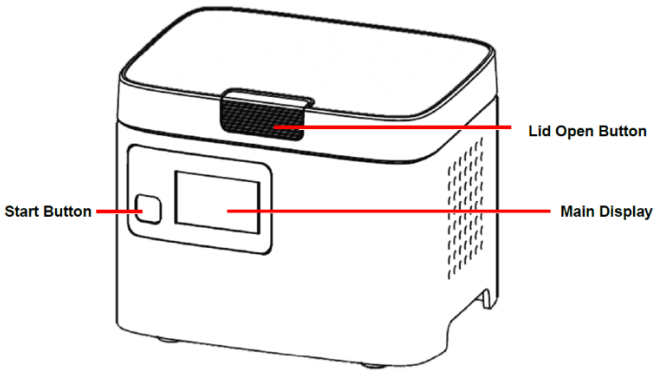
2. After the PCR is complete, transfer the PCR tube to the CGE system for analysis.

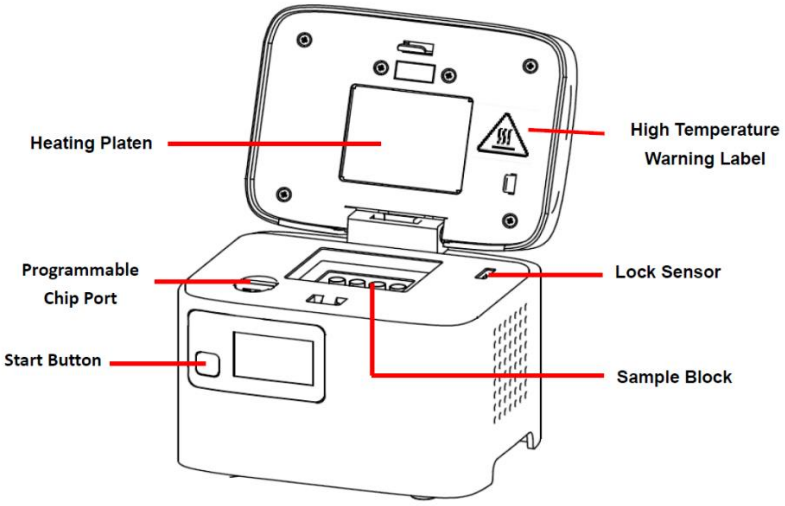
Perform PCR using *Qamp*$_{mini}$™ Thermal Cycler

General Description

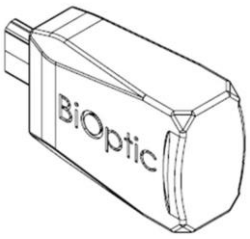
Before using the *Qamp*$_{mini}$™, please read this operation manual carefully and pay attention to the safety information. To guarantee problem-free operation, please follow the instructions and safety precautions to ensure the safe operation of the *Qamp*$_{mini}$™.

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 its 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 5 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 6 Instrument and software compatibility

Instrument	*Q-Analyzer*™
Qsep$_{400}$™ Bio-Fragment Analyzer	Version 4.0.1 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 7 Software Requirements

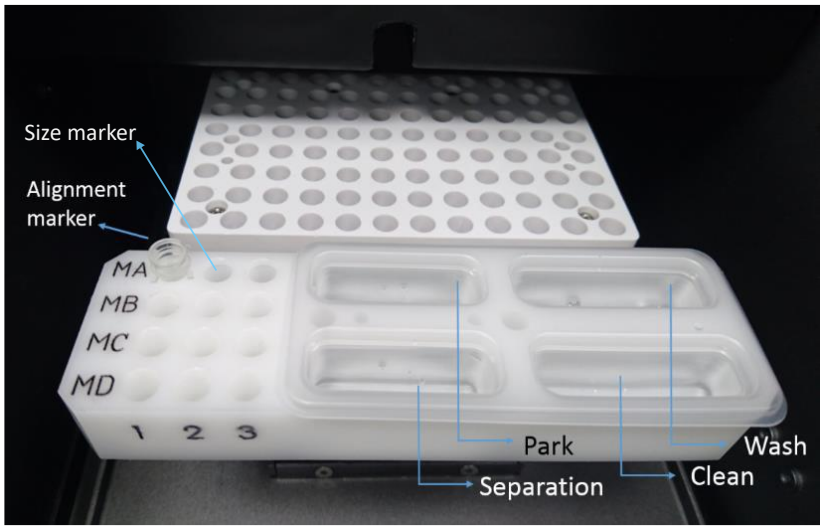
	Minimum	Recommended
OS	Windows 10 32 bit	Windows 10 64 bit
CPU	Intel Core i5	Intel Core i7
RAM	8G RAM	16G RAM
HDD	500 GB	1 TB
PORT	RJ45 x 1 USB 3.0 x 1	RJ45 x 2 USB 3.0 x 2
Screen Resolution	1280 x 800	1920 x 1080

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, Size Marker (C109200) at MA2.

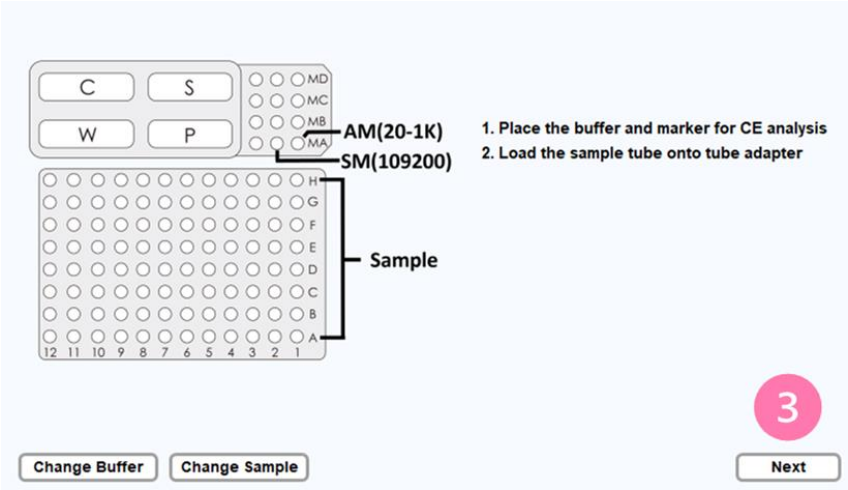


   "Change Sample" to place the samples. Remember to remove the cap of PCR tube. (For more information, please refer to *Qsep* Series Operation Manual)

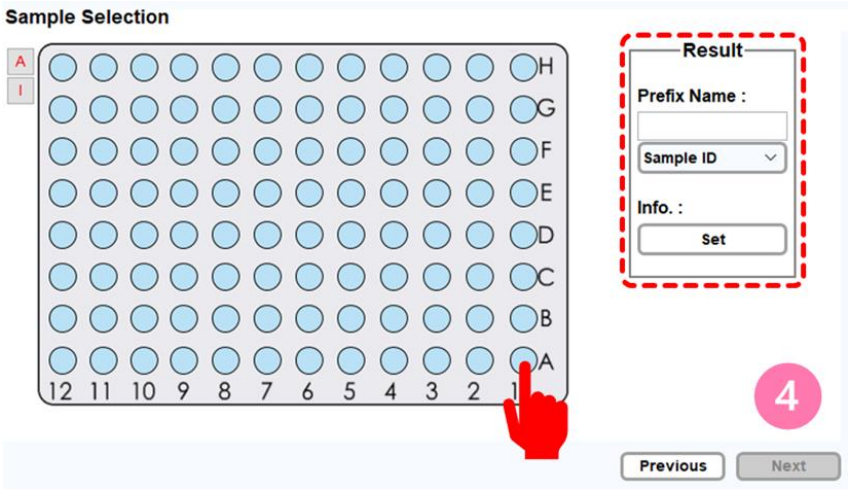
2. **"Latch"** the S1 cartridge. (For more information, please refer to the F0060-S1_ High Resolution Cartridge User Guide)

3. Switch to **"Assay"** and select the select the icon in the following order: **"MDx"** → **"Metabolism"** → **"ADRB3"** .

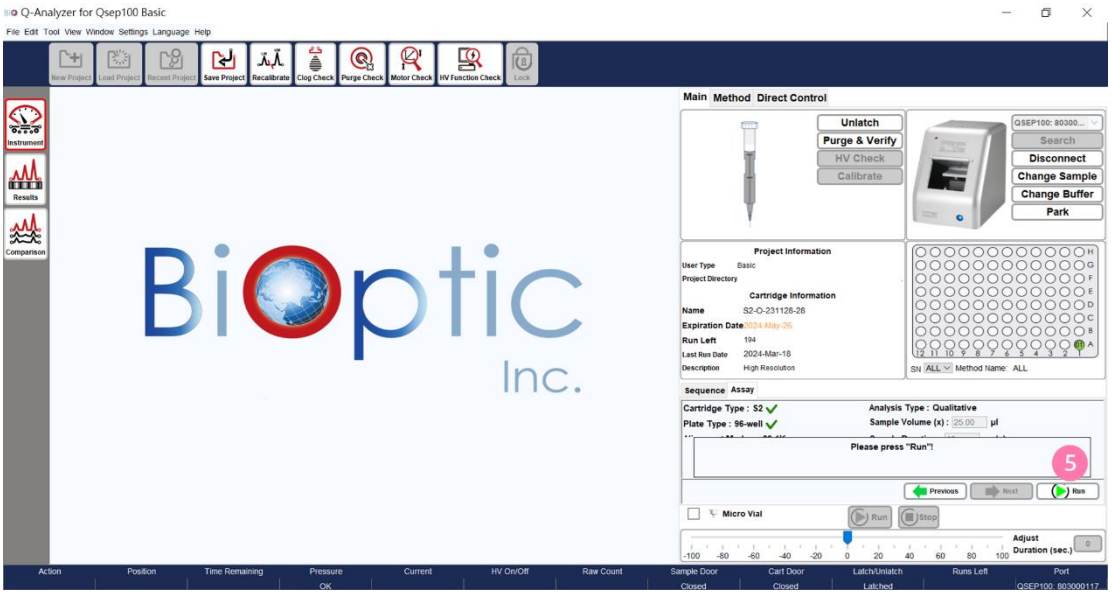
4. Click **"Next"** to select the sample position.



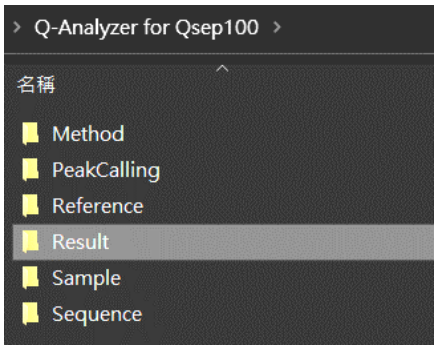
5. Select the sample position and click **"Next"** .
6. (Optional) Type the result name in the field of **"Prefix Name"** and click **"set"** to type the desired information of the sample.



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



5. 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 must be contaminated in the procedure.
- Ensure that all signals of the target sequences are shown in the positive control.
  - The signal pattern of the positive control should be like that of medium risk.
  - If the positive control did not show any signal, it may be caused by the degradation of the reagents. Please contact the distributor in your area.

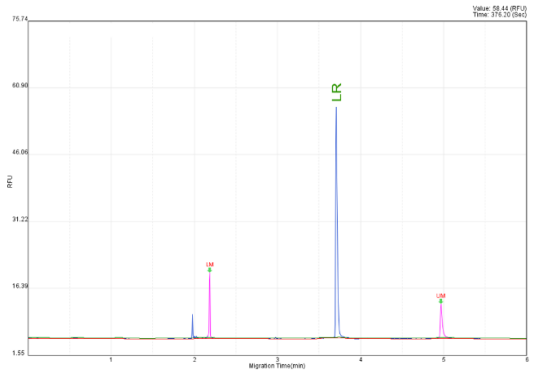
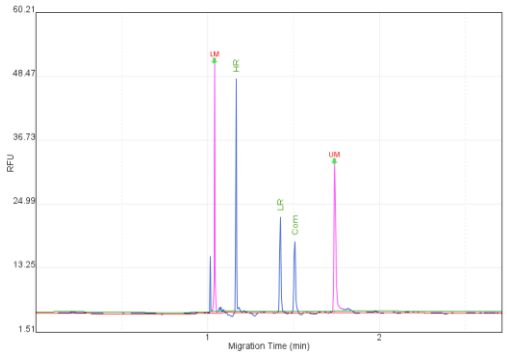
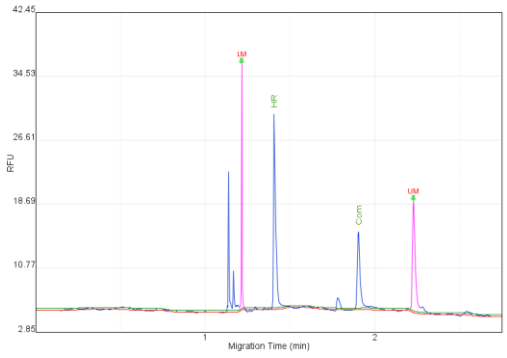
The result of Direct ADRB3 assay

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

Table 8 The size of the target sequences

		High Risk (HR)	Low Risk (LR)	Common (Com)
Target size	ADRB3	98 bp	252 bp	312 bp

Table 9 The CE result and interpretation of Direct ADRB3 kit assay

Type	Result data
Low Risk	
Medium Risk	
High Risk	

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 10 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