For Professional Use Only

Obesity & Diabetes Screen

Handbook

Real Time PCR test for dection of 8 single nucleotide polymorphisms (SNPs): PPARG2, ADRB2, ADRB3, FABP2, LPL, INS and FTO genes

REF T01372-96-S

Σ 12

NAME

Obesity & Diabetes Screen

INTRODUCTION

Obesity is one of the most prevalent disorders of developed countries, and its incidence is increasing rapidly. Obesity in general is associated with physiological changes that may cause the development of diseases like high blood pressure, heart disease, high blood cholesterol and type 2 diabetes.

Diabetes is defined as the inability of the body to regulate and control the blood sugar levels. Persistent and long term raised blood sugar is associated with damage to blood vessels, nerves and several organs.

INTENDED USE

Obesity & Diabetes Screen kit is intended for detection and allelic discrimination of genetic polymorphisms associated with susceptibility to increased risk of diseases associated with obesity and diabetes.

PRINCIPLE OF ASSAY

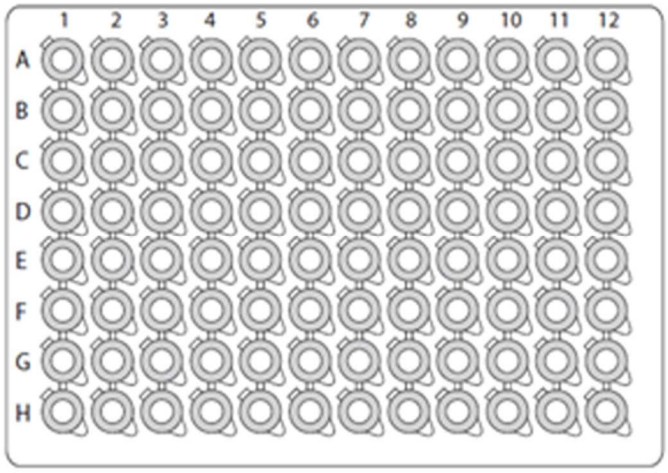
Obesity & Diabetes Screen kit is a qualitative test that allows the detection by Real Time PCR based on the amplification of the genome specific region using specific primers. In Real Time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product. The real-time monitoring of the fluorescence intensities during the reaction allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. One dye (HEX) will detect wild type sequence, the other one (FAM) the mutant sequence. If a patient has positive signal in both fluorescence channels, it means it's heterozygous genotype. If patient has positive signal only in FAM channel, will be considered carrier of mutant genotype for the target gene/region. If patient has positive signal only in HEX channel, will be considered normal (wild-type) genotype for the target gene/region.

MATERIALS PROVIDED

Part N°1: ready to use 12x8 strip with PCR mix

- **12 x 8 strip ready to use** (each PCR tube contains 15 µl of PCR mix):

  PCR mix **PPARG2 Pro12Ala**
  PCR mix **ADRB2 Gln27Glu**
  PCR mix **ADRB2 Arg16Gly**
  PCR mix **ADRB3 Trp64Arg**
  PCR mix **FABP2 Ala54Thr**
  PCR mix **LPL HindIII**
  PCR mix **INS 23HphI**
  PCR mix **FTO A23525T**



A - PPARG2 Pro12Ala ref. 335
B - ADRB2 Gln27Glu ref. 358
C - ADRB2 Arg16Gly ref. 359
D - ADRB3 Trp64Arg ref. 360
E - FABP2 Ala54Thr ref. 361
F - LPL HindIII ref. 397
G - INS 23HphI ref. 398
H - FTO A23525T ref. 329

Part N°2: controls and enzyme

- **Diluent** 0.20 ml (1 vial)
- **Taq-polymerase** 0.55 ml (1 vial)
- **Positive control samples C+** (3 vials each):

Positive Control	Allele 1-1 homozygous wild type	Allele 1-2 heterozygous	Allele 2-2 homozygous mutant
C+ **PPARG2 Pro12Ala** (ref. 335)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)
C+ **ADRB2 Gln27Glu** (ref. 358)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)
C+ **ADRB2 Arg16Gly** (ref. 359)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)
C+ **ADRB3 Trp64Arg** (ref. 360)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)
C+ **FABP2 Ala54Thr** (ref. 361)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)
C+ **LPL HindIII** (ref. 397)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)
C+ **INS 23HphI** (ref. 398)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)
C+ **FTO A23525T** (ref. 329)	60 µl (1 vial)	60 µl (1 vial)	60 µl (1 vial)

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation

- DNA extraction kit
- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes
- Dry heat block
- Vortex mixer
- Pipettes
- Sterile pipette tips with filters
- 1,5 ml polypropylene sterile tubes
- Biohazard waste container
- Refrigerator, Freezer

Zone 2: Real Time amplification

- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes
- Freezer, refrigerator
- Tube racks

STORAGE INSTRUCTIONS

Obesity & Diabetes Screen kit must be stored at 2-8°C. The kits can be shipped at 2-8°C and stored as indicated immediately on receipt.

STABILITY

Obesity & Diabetes Screen kit is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

QUALITY CONTROL

In accordance with Sacace’s ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

RUO For Research Use Only

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

PRODUCT USE LIMITATIONS

Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Obesity & Diabetes Screen Kits can analyze genomic DNA extracted from:

- *whole blood* collected in EDTA tubes;
- *buccal swab*: insert the swab into the nuclease-free 1,5 ml tube and add 0,2 ml of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.

Specimens can be stored at +2-8°C for no longer than 24 hours, or freeze at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

The following isolation kits are recommended:

⇒ **Genomic column DNA Express** – spin column extraction kit (Sacace, REF K-1-1/E)

⇒ **SaMag Blood DNA extraction kit** (Sacace, REF SM001);

⇒ **QIAamp DNA Blood mini kit** (Qiagen, REF 51104);

⇒ **DNA-Sorb-A** (Sacace, REF K-1-1/A) for buccal swab;

Please carry out DNA extraction according to the manufacturer's instruction.

PROTOCOL

Obesity & Diabetes Screen kit does not include reagents required for sample preparation and DNA extraction. Blood samples and biological materials must be processed by using the recommended kits or those with similar performances of quality and quantity of extracted DNA. Use of blood samples collected in tubes containing heparin is not recommended.

The analysis of the genomic DNA specimens using **Obesity & Diabetes Screen** kit includes the following stages:

1. Preparing the Real Time PCR;
2. Real Time PCR analysis;
3. Data analysis with the software of Real Time PCR instrument;
4. Results analysis and conclusions.

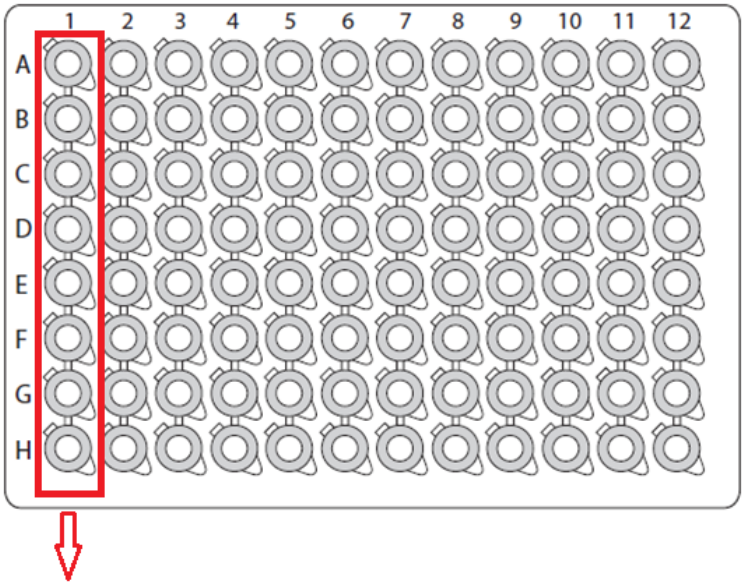
EXPERIMENTAL PROTOCOL

Total reaction volume: 25 µl

1. Prepare the necessary number of ready-to-use strips (1 eight tube strip for each clinical sample) and mark them.
2. Spin for 3-5 sec the **Taq polymerase**, mix by pipetting and **add 5 µl** to each PCR tube.
3. Add into the corresponding PCR tubes strip **5.0 µl** of extracted DNA from clinical sample.
4. Add into the corresponding PCR tubes strip **5.0 µl** of controls according to the related PCR mix:
    - **C+ Homozygous Wild Type (allele 1-1)**
    - **C+ Heterozygous (allele 1-2)**
    - **C+ Homozygous Mutant (allele 2-2)**

** it's suggested to use at least 1 Positive control (for example C+ Heterozygous) for each run.*

5. Spin the tubes for 3–5 seconds to collect the drops.
6. Insert the tubes in the Real-time PCR instrument.



A – **PPARG2** Pro12Ala

B - **ADRB2** Gln27Glu

C - **ADRB2** Arg16Gly

D - **ADRB3** Trp64Arg

E - **FABP2** Ala54Thr

F - **LPL HindIII**

G - **INS 23HphI**

H – **FTO** A23525T

NOTE: *use 1 eight tube strip for each clinical sample. Add 5 µl of Taq polymerase and then 5 µl of extracted DNA to each tube of the strip*

(e.g. sample 1 DNA in tubes A1,B1,C1,D1,E1,F1,G1,H1

sample 2 DNA in tubes A2,B2,C2,D2,E2,F2,G2,H2)

Amplification

Create a temperature profile on your instrument* as follows:

	Temp °C	Time (s)	Cycles
Hold	80	2 min	1
Hold	94	3 min	1
Cycling	94	15 sec	5
	64	40 sec	
Cycling	94	15 sec	35
	64	**40 sec fluorescence detection**	

* *For example,SaCycler-96™ (Sacace), CFX-96 / iQ5™ (BioRad); Mx 3000P/3005P™ (Agilent); ABI® 7300/7500 Real Time PCR (Applied)***

*** To perform the test with ABI 7500 (Applied) a disposable adapter provided with the kit has to be used. Additional adapters can be purchased separately.*

Fluorescence is detected in FAM/Green (allele 2), JOE/Yellow/HEX (allele 1) fluorescence channels.

DATA ANALYSIS

The fluorescent signal intensity is detected in 2 channels as shown in the table below:

FAM	HEX/Joe
Allele 2 (mutant)	**Allele 1 (wild type)**

The fluorescent signal is detected as shown in the table below:

Gene	Polymorphism		SNP ID	Allele		Channel
PPARG2	Pro12Ala	C34G	rs1801282	Pro (C)	Allele 1	Yellow/HEX
				Ala (G)	Allele 2	Green/FAM
ADRB2	Gln27Glu	5318C>G	rs1042714	Gln (C)	Allele 1	Yellow/HEX
				Glu (G)	Allele 2	Green/FAM
ADRB2	Arg16Gly	46A>G	rs1042713	Arg (A)	Allele 1	Yellow/HEX
				Gly (G)	Allele 2	Green/FAM
ADRB3	Trp64Arg	190T>C	rs4994	Trp (A)	Allele 1	Yellow/HEX
				Arg (G)	Allele 2	Green/FAM
FABP2	Ala54Thr	163G>A	rs1799883	Ala (G)	Allele 1	Yellow/HEX
				Thr (A)	Allele 2	Green/FAM
LPL	HindIII	T495G	rs320	T	Allele 1	Yellow/HEX
				G	Allele 2	Green/FAM
INS	-23HphI	A>T	rs689	A	Allele 1	Yellow/HEX
				T	Allele 2	Green/FAM
FTO		A 23525 T	rs9939609	A	Allele 1	Yellow/HEX
				T	Allele 2	Green/FAM

Interpretation of results for CFX96/iQ5 (Bio-rad):

Principle of interpretation:

- Signal only in allele 1 (channel HEX) : homozygous wild type
- Signal only in allele 2 (channel FAM) : homozygous mutated
- Signal in both allele 1 and allele 2 (channels HEX and FAM) : heterozygous

Set **Cycles to Analyze** at 6-35 for all samples and **Crossing Threshold** value as ~100 for both channels. Choose "Allelic Discrimination" Analysis mode.

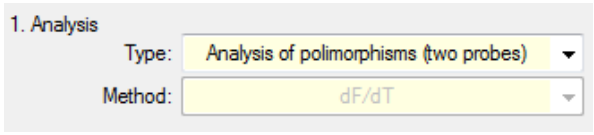
NOTE FOR CFX96 / iQ5 (Bio-rad): if a Ct value is higher than 32 the sample is considered invalid and must be repeated. If there is no Ct value in both channels the sample is invalid and must be repeated starting from the extraction.

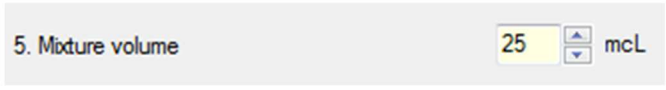
Interpretation of results for SaCycler-96 (Sacace Biotechnologies):

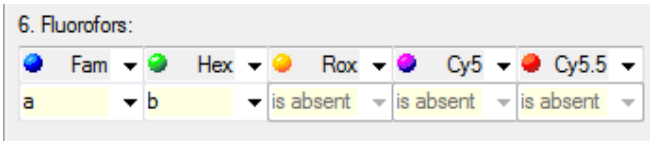
Principle of interpretation:

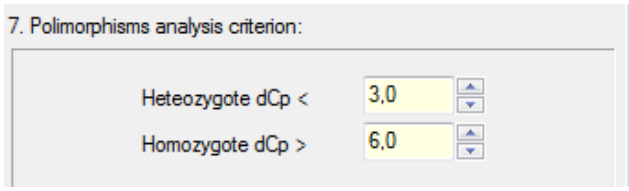
- Signal only in allele 1 (channel HEX) : homozygous wild type
- Signal only in allele 2 (channel FAM) : homozygous mutated
- Signal in both allele 1 and allele 2 (channels HEX and FAM) : heterozygous

NOTE: when creating new test, select **"Analysis of polimorphisms (two probes)"**, name "a" on FAM channel and name "b" on HEX channel. Set **Heterozygote dCp < 3,0** and **Homozygote dCp > 6** (see pictures below).

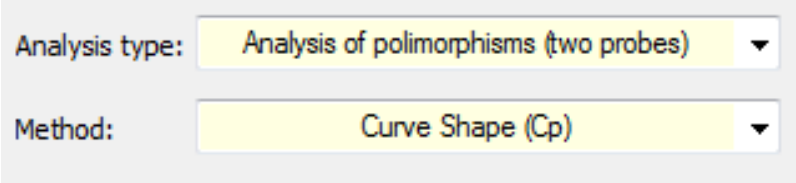








To analyse results, be sure to select **"Analysis of polymorphisms (two probes)"** as Analysis type and **"Curve Shape (Cp)"** as Method.



The results will be displayed in the table on the right (see below pictures as reference).

Example of results:

Results | Statistics

N	Identificator	Polimorphism		dCp	Cp Fam	Cp Hex
A1	Sample_1_	a	b	0,2	19,2	19,1
A2	Sample_2	b	b	>17		17,7
A3	Sample_3	b	b	>16		18,3
A4	Sample_4	b	b	>16		18,8
A5	K+	b	b	>15		19,4
A6	K+	a	b	0,1	18,9	19,0
A7	K+	a	a	>16	18,4	
A8	C_-	-	-			

a = FAM (mutant, allele2) **b = HEX (wild type, allele1)**

a | b **= sample heterozygous (both alleles present)**

b | b **= sample homozygous wild type (only allele 1 present)**

a | a **= sample homozygous mutant (only allele 2 present)**

NOTE FOR SaCycler-96 (Sacace Biotechnologies): if a Ct value is higher than 32 the sample is considered invalid and must be repeated. If there is no Ct value in both channels the sample is invalid and must be repeated starting from the extraction.

For channels FAM and HEX use following recommended threshold settings:

- Channel HEX **Threshold** ~20
- Channel FAM **Threshold** ~200

Software automatically analyzes the results.

KEY TO SYMBOLS USED

REF	List Number		Caution!
RUO	For Research Use Only		Contains sufficient for <n> tests
	Expiration Date	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**IC**	Internal Control
LOT	Lot Number		

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* iQ5™ is a registered trademark of Bio-Rad Laboratories
* MX3005P® is a registered trademark of Agilent Technologies
*ABI® is a registered trademark of Applied Biosystems

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314892926
mail: ***info@sacace.com*** *web:* ***www.sacace.com***