



## Ppar $\gamma$

Detection system of the PPAR $\gamma$  Pro12Ala variant in the PPAR $\gamma$ -2 gene



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Ppar  $\gamma$

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### Kit Utility

The kit analyzes the presence of the mutation Pro12Ala in codon 12 of the gene that encodes for the receptor PPAR $\gamma$ -2.

### Principle of the Assay

The analysis for the detection of the Pro12Ala mutation in the PPAR $\gamma$ -2 gene implicates a PCR amplification of the segment that contains the mutation.

The presence or absence of a base substitution is later detected by RFLP.

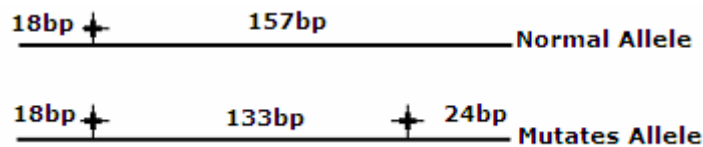
There are three possible test results:

- Homozygote Pro/Pro, when the Pro12Ala mutation cannot be detected in neither of both alleles.
- Heterozygote Pro/Ala, when the Pro12Ala mutation is detected in only one the alleles.
- Homozygote Ala/Ala, when the Pro12Ala mutation is detected in both alleles.

### Introduction

The PPARs are part of sub-family of transcriptional factors: the nuclear hormonal receptors. PPAR $\gamma$ -2, which gene is located in chromosome 3, is mostly present in the adipose tissue and has a role in the differentiation and function of the adipocytes. Pro12Ala is a frequent polymorphism of the PPAR $\gamma$ -2 gene and has been associated to an increased susceptibility to obesity, higher body mass index and type 2 diabetes. It is also, one of the best genetic predictors of weight gain.

### Experimental strategy:



### Kit presentation

Color that identifies the kit: yellow

The ATGen kit for the detection of the G>C (Pro12Ala) mutation in the PPAR $\gamma$ -2 gene includes:

- 1 PPAR $\gamma$  Reaction Mix tube.
- 1 PPAR $\gamma$  Restriction Enzyme tube.
- 1 PPAR $\gamma$  Positive Control DNA tube, containing heterozygote control DNA (once defrosted it is recommended to keep it at 4 °C).
- 1 PPAR $\gamma$  Taq DNA polymerase tube.



- 1 PPAR $\gamma$  Molecular Weight tube, containing 3 bands: the amplification product and the two possible digestion bands. This tube should be kept in the post-amplification zone if possible.

The kit must be kept at -20 °C.

Our kits are available in two sizes: 20 and 50 reactions.

### Necessary materials not provided with the test

- DNA-free PCR tubes
- Suitable pipettes
- Filtered pipette tips
- Gloves and robe
- Thermocycler
- Cuvette for vertical electrophoresis
- Acrylamide, electrophoresis buffer and loading buffer
- Gel coloring system with silver nitrate.
- Vortex
- Bio-hazard disposable container

### Precautions

1. Only for in-vitro use.
2. All samples, reagents and controls should be considered potentially infectious.
3. Do not use after the expiration date indicated on the package.



### Storage and Stability

The kit must be stored at -20°C in order to assure its optimal performance through the expiration date indicated on the package.

### Specimen Characteristics

The sample must be a DNA solution with a 50-100 ng /  $\mu$ l concentration, apt for PCR amplification.

ATGen recommends obtaining the DNA from blood specimens by using ADN Facil kit.

### Protocol

Defrost the reaction mix and shake it vigorously by vortexing.

If possible, perform all manipulations in cold room.

### Preparation of the amplification mix:

- 1. Add 18  $\mu$ l of reaction mix per sample to be tested.
- 2. Add to the reaction mix, 1  $\mu$ l of DNA Taq polymerase per sample to be tested.
- 3. Homogenize by moderate vortexing or pipetting.



It is recommended to prepare a single amplification mix containing the necessary quantities of reaction mix and DNA Taq polymerase, according to the number of samples to be analyzed.

It is necessary to add two reactions, one for the positive control and one for the negative control.

### **Amplification:**

- 4. Aliquot the amplification mix, dispensing 18  $\mu$ l in properly labeled PCR tubes.
- 5. Add 2  $\mu$ l of sample to each tube.

The samples must contain between 100 and 200 ng of DNA (we recommend to use ATGen's ADN Facil Kit for the DNA extraction).

- 6. Add 2  $\mu$ l of PPAR $\gamma$  control DNA to the positive control tube and 2  $\mu$ l of the water previously used to dissolve the sample DNA, to the negative control tube.
- 7. Run the program for PPAR $\gamma$ .

Program PPAR $\gamma$ : 35 cycles at 94  $^{\circ}$ C/0:30', 56  $^{\circ}$ C/0:30', 72  $^{\circ}$ C/0:30'; an initial denaturing step: 3 minutes at 94  $^{\circ}$ C and a final extension step: 5 minutes at 72  $^{\circ}$ C.

- 8. Place the tubes into the thermocycler when it reaches 94 $^{\circ}$ C.

Once the program has ended and in the case you are not going to immediately perform the next step, keep the tubes at 4 $^{\circ}$ C until the digestion stage.

Optionally, the amplification can be tested by electrophoresis by loading 5  $\mu$ l of the reaction product and using a 6% acrylamide gel.

The expected size of the amplification product is 175 bp.

### **Digestion:**

- 9. Once the program has ended allow the temperature to drop until the tubes reach room temperature and then add 1  $\mu$ l of restriction enzyme to every amplification tube.
- 10. Homogenize using the pipette.
- 11. Incubate for 2:30 hrs at 37  $^{\circ}$ C (it is also possible to incubate overnight).



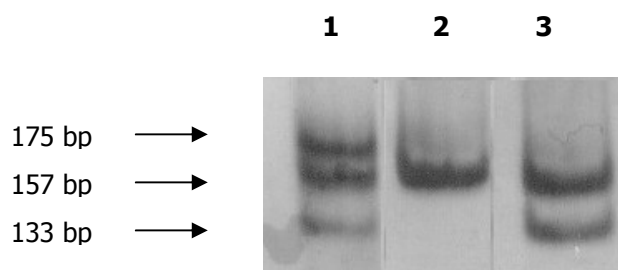
### Obtention of test results

1. Prepare samples with the indicated quantity of an adequate loading buffer (e.g. glycerol 30% p/v, xilencianol blue 0.25% p/v, bromophenol blue 0.25% p/v).
2. Load 5 µl of each digested amplification product and 5 µl of PPAR $\gamma$  molecular weight marker in a 6% acrylamide gel or 20 µl of each one in a 2% agarose gel, pre-stained with ethidium bromure (0.5 µg/ml).
3. Migrate the bromophenol blue (of the loading buffer): 8 cm in acrylamide or 3.5 cm in agarose.
4. Use silver nitrate for staining the acrylamide or use uv light for viewing the agarose gel.

### Interpretation of the results

Electrophoretic pattern	Result
133 bp	Mutated Homozygote Ala/Ala
157 + 133 bp	Heterozygote Pro/Ala
157 bp	Normal Homozygote Pro/Pro

### Example:



6% Acrylamide gel showing the possible results:

Lane 1 shows the three bands corresponding to Ppar  $\gamma$  (175, 157 and 142 bp bands).  
 Lane 2 shows the result corresponding to a normal Pro/Pro homozygote individual for the Ppar  $\gamma$  mutation (157 bp band).  
 Lane 3 shows the result corresponding to a Pro/Ala heterozygote individual for the Ppar  $\gamma$  mutation (157 and 133 bp bands).  
 It is important to check that the 175 bp disappears after the enzymatic digestion, indicating digestion was complete.



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## **Bibliography:**

1. OMIM \*601487 Peroxisome Proliferator-Activated Receptor-Gamma; pparg
2. Pisabarro R, Stoll M, Sanguinetti C., Prendez D. High incidence of type 2 Diabetes in PPAR  $\gamma$ 2 Pro12Ala carriers exposed to a high chronic intake of Trans fatty acids and saturated fatty acids. Diabetes Care 2004, Vol 27, 2251-2252.

