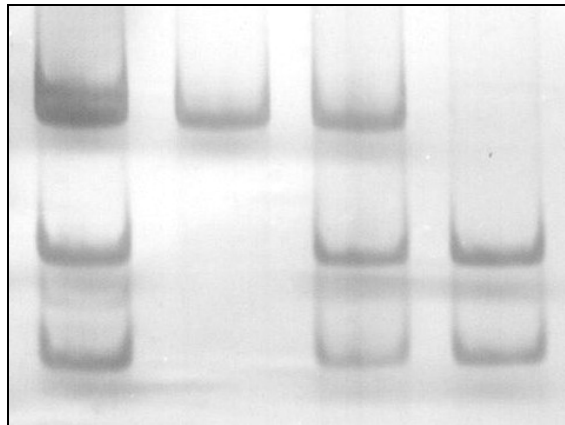




## Protein G – C825T

Detection system of the C825T mutation in the Protein G gene



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**Protein G**

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### **Kit Utility**

The kit analyzes the presence of a polymorphism in the position 825 of exon 10 of the gene that encodes for human Protein G.

### **Principle of the Assay**

The analysis for the detection of the C825T mutation in exon 10 of the Protein G gene implicates a PCR amplification of the segment containing the polymorphic site. The presence or absence of a base substitution is later detected by RFLP.

There are three possible results:

Homozygote CC, when there is no detection of the C825T mutation in neither one of the gene alleles.

Heterozygote CT, when the C825T mutation can only be detected in one of both gene alleles.

Homozygote TT, when the C825T mutation is detected in both gene alleles.

### **Introduction**

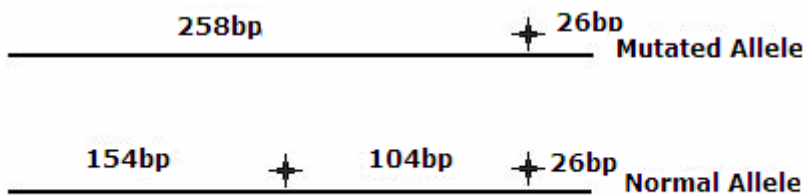
The GNB3 gene, located in chromosome 12, codifies for the Gβ3 sub-unit of Protein G.

The C825T polymorphism in exon 10 is associated to a splicing variant which loses the nucleotides 498 and 620 in exon 9 of the primary transcript and to the loss of 41 amino acids in the encoded protein.

Protein G belongs to a certain type of cellular receptors, which act as signal intracellular transducers, which are targeted by several drugs, thus playing an important role in its pharmacological action.

It has been demonstrated the association of this polymorphism with obesity, weight gain during pregnancy and hypertension. The response to sildenafil citrate in patients with erectile dysfunction was reported to diminish in CC and CT genotypes.

### **Experimental Strategy:**



**Protein G**

## Kit presentation

The Protein G C825T kit includes:

- 1 Protein G Reaction Mix tube.
- 1 Protein G Restriction Enzyme tube.
- 1 Protein G DNA Positive Control containing heterozygote control DNA (once defrosted it is recommended to keep it at 4 °C).
- 1 Protein G Taq DNA polymerase tube.
- 1 Protein G Molecular Weight tube, containing 4 bands which correspond to the amplification product and the three 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 / µl concentration, apt for PCR amplification.

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

## Protocol

### *Pre-amplification zone*

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 µl of reaction mix per sample to be tested.
- 2. Add to the reaction mix, 1 µl 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 µl in properly labeled PCR tubes.
- 5. Add 2 µl 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 µl of Protein G control DNA to the positive control tube and 2 µl of the water previously used to dissolve the sample DNA, to the negative control tube.
- 7. Run the program for Protein G

Program for Protein G C825T: 35 cycles at 95 °C/0:30'; 60 °C/0:30'; 72 °C/0:30' an initial denaturizing step: 5 minutes at 95 °C and a final extension step: 5 minutes at 72°C.

- 8. Place the tubes into the thermocycler when it reaches 94°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°C until the digestion stage.

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

The expected size of the amplification product is 284 bp.

### Digestion:

- 9. Once the program has ended allow the temperature to drop until the tubes reach room temperature and then add 1 µl of restriction enzyme to every amplification tube.



**Protein G**

- 10. Homogenize using the pipette.
- 11. Incubate for 3 hrs at 60 °C (it is 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 Protein G molecular weight marker in a 10% acrylamide gel.
3. Migrate the bromophenol blue (of the loading buffer) until it reaches the end of the gel.
4. Use silver nitrate for staining the acrylamide.

### Interpretation of the results

<b>Test</b>	<b>Homozygote CC</b>	<b>Heterozygote CT</b>	<b>Homozygote TT</b>
Protein G C825T	154 + 104 bp	258 + 154 + 104 bp	258 bp

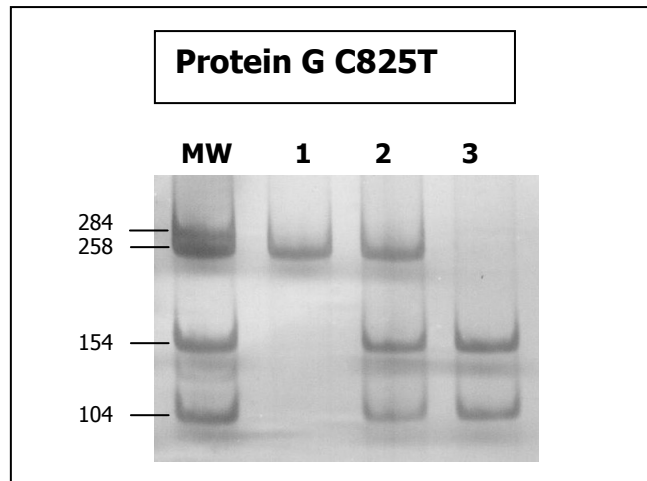
#### Note:

The band corresponding to the amplification product must not appear after digestion. In case this 284 bp band appears, the digestion was no complete.



**Protein G**

**Example:**



10 % acrylamide gel stained with silver nitrate, showing the possible test results:

1: Homozygote TT

2: Heterozygote CT (The Protein G control DNA must show this result)

3: Homozygote CC

MW: Protein G Molecular weight marker showing the amplification band and all the possible digestion bands.

**Bibliography**

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