

IDENTIFICATION OF SERT POLYMORPHISM (SLC6A4) IN 5-HTT GENE

AMPLI-SET SERT (SLC6A4)

Cat. N. 2.113

The SERT polymorphism (SLC6A4) is the most common in the insert region of 5-HTT gene promoter. This polymorphism is considered to be bi-allelic: long allele (L) and short allele (S). The S variant is associated with a poor expression of 5-HTT and, therefore, a poor 5-HT re-uptake efficiency.

5-HTT is the gene that carries the human serotonin (5-HTT, SLC6A4, o SERT), and it has been cloned and sequenced. The maps of chromosome 17q11.1-q12, as monoamine carriers, are organized in 14 exons able to produce a protein with 12 membrane domains. The 5-HTT gene codes for a serotonin carrier protein and it is very active in the neural pathways. It is now known that these pathways are involved in the mood, emotions, aggressiveness, sleep, anxiety control and they are responsible for the serotonin uptake in the pre-synaptic cell, as the serotonin has been released in the synaptic space for the signal to the next neuron. This protein is known to be in the cell pre-synaptic membrane. The activity and the number of the serotonin carrier proteins determines the residence time of the chemical signal in the synapse (Glatz, et. Al. 2003). The Ampli set SERT (SLC6A4) kit is able to identify the gene 5-HTT polymorphism by using PCR technique in three steps: DNA extraction, Amplification and revelation on Pre-cast agarose gel.

Principle of method A) genomic DNA extraction B) amplification C) enzymatic digestion D) revelation on agarose gel.

Applicability: on purified and extracted genomic DNA from whole blood and mouth swab.

Number of Test: 50

KIT CONTENT AND ITS STORAGE

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| AMPLIFICATION | |
| Mix PCR | -20°C |
| Taq Polymerase (5U/μl) | -20°C |
| Sterile H ₂ O | -20°C |
| Positive control | -20°C |
| REVELATION | |
| Agarose Gel di agarosio 4% for electrophoresis in TBE 1x | T.A. |
| Loading buffer 10 X | T.A. |
| Running buffer 5 X (TBE 5X) | T.A. |
| Marker with molecular weight ladder 100 bp | -20°C |

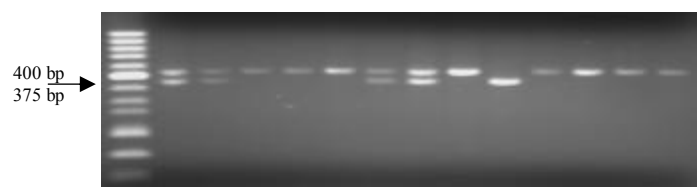
stability: over 12 months if correctly stored (in the dark at room temperature)

requested materials: 1,5 ml tubes; 15 ml tubes in polyarylene; glass rod; refrigerated tubes holders; sterile tubes with anti-aerosol barrier; PCR tubes .

Requested instrumentation: centrifuge with fix rotor for 2ml tubes (12.000 x g) and centrifuge with oscillating rotor for 15ml tubs (1.500 x g), pipettes' Set di pipette pre-PCR and post-PCR (0.5 – 20 μl, 10 – 100 μl, 20 – 200 μl, 200 – 1000 μl); programmable thermocycler Biohazard cabinet class II; electrophoresis with power supply, trans-illuminator UV; photo equipment.

All the materials to the execution of the test must be sterile, DNase and RNase free and single-use.

Ladder 50bp S/L S/L L/L L/L L/L S/L S/L L/L S/S L/L L/L L/L L/L



Picture of 2% gel of the amplified 5HTTLPR DNA . Lane 1 ladder 50 bp, with reference band of 400 bp. The primer pair amplifies 400bp for 16-repeat “long” (L) and amplifies 375 bp for 14-repeat “short” (S)

REFERENCES:

- Fumeron F,** (2001) Association of a functional 5-HT transporter gene polymorphism with anorexia nervosa and food intake. Mol Psychiatry 6 (1):9.
Monteleone P, (2005) Association of a functional serotonin transporter gene polymorphism with binge eating disorder. Am J Med Genet B Neuropsychiatr Genet 141B (1):7-9.