Pineta Grande Hospital
Castel Volturno

dia-chem s.r.l.
DIAGNOSTICI E TECNOLOGIE BIOMEDICHE
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CE

Detection of Val762Ala polymorphism of PARP-1 (Poly-ADP-ribose)-polymerase-1 enzyme

AMPLI-set Val762Ala PARP-1 Cat. n.1.429

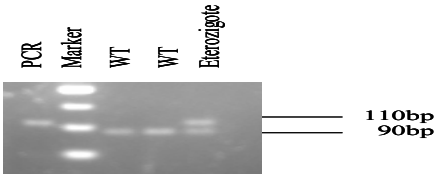
Genomic DNA is often damaged exogenously and endogenously. The unrepaired damage may cause apoptosis or may lead to unregulated cell growth and thus to cancer. The damage may be repaired in DNA allowing the regular replication of cells. Due to the importance of keeping genomic integrity, genes encoding for DNA repairer molecules has been purposed as genes affecting the predisposition to cancer. *Poly (ADP-ribose) polymerase* (PARP-1) is an enzyme involved in repair of DNA and it catalyzes poly-(ADP- ribosilation) of protein using NAD+ as substrate. The Poly-ADP-ribosilation is involved in many cell process as replication, transcription, keeping genomic stability and regulation of cell cycle and cell differentiation. This enzyme may inhibit cancer progression, promoting genomic stability, repairing DNA and controlling cell cycle. Consistent with this view, the presence of polymorphism reducing the PARP activity may represent a risk factor for cancer growth. Recently, a polymorphism in PARP gene has been identified, T2444C substitution, which causes the substitution of Valine amino acid with an Alanine (Val762Ala). This amino acidic change causes a reduced activity of PARP protein. This polymorphism has been associated to an increased tendency to prostate, esophagus and lung cancer. The detection of T2444C mutation is performing by amplification with specific primers of a 110bp fragment, followed by a restriction digestion with HpyCH4-I enzyme. T-C substitution produces the loss of a restriction site. The digestion product of wild type allele produces two fragments 90 and 20bp , whereas mutant allele is not digested (110bp)

REAGENTS and STORAGE

AMPLIFICATION and DIGESTION	
PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
HpyCH4-IV (10 U/µl) Enzyme	-20°C
Digestion BUFFER 10X	-20°C
WT CTR	-20°C

Principle of method:
A) extraction of genomic DNA
B) amplification
C) enzymatic digestion
D) detection on agarose gel
Applicability: On extracted and purified genomic DNA from fresh and fixed tissues
Test: 24 reactions
Stability: over 18 months if correctly stored.

ANALYSIS OF RESULTS



WT	ETERO	OMO
	110	110
90	90	
20	20	