

DETECTION OF AZF REGIONS MICRODELETIONS of Y CHROMOSOME

AMPLI-SET Y Cromosoma UE FAM

Cat. n.1.501FAM

Y Chromosome microdeletions of AZF regions (AZoospermia Factor) are observed in 10-15% of azoospermic men and 5-7 % of oligospermic ones. Many genes in every AZF region have been identified (DBY, USP9Y RBMY1, eIF1AY, DAZ, GOLG, BPY2 etc), but it isn't clear which of them is involved in spermatogenesis. Deletions of regions of the long arm of Y Chromosome can occur and partial microdeletions or deletions of single genes are rare (1,2).

The ampli-set Y Chromosome UE allows the detection, using the Polymerase Chain Reaction (PCR), of Y chromosome microdeletions inside the three AZF regions (AZoospermia Factor) AZFa, AZFb, AZFc. The Multiplex PCR mix (First Step M-PCR) amplifies the "sequence tagged sites" (STS) assessed by the Guide Lines of the European Academy of Andrology (3) and showing almost 100% of the deletions having clinical significance. The deletion is showed by the absence of PCR product. The mix PCR contains a specific primers pair for ZFX/ZFY genes, that always produces an amplification product (internal PCR product), and a specific primers pair for SRY gene on the short arm of Y chromosome as "testis determining factor" control.

Principle of Assay: A) extraction of genomic DNA B) amplification C) detection on agarose gel.

Applicability: On extracted and purified genomic DNA from whole blood samples.

Numbers of Tests: 24

REAGENTS and STORAGE

AMPLIFICATION	
M-PCR mix A	-20°C
M-PCR mix B	-20°C
H ₂ O sterile	-20°C
Taq Polymerase (5U/μl)	-20°C
Healthy male DNA	-20°C

Stability: over 12 months if correctly stored (Agarose gels, if protected by light, can be stored 1 year at room temperature).

M-PCR mix A	M-PCR mix B
ZFY 495 bp	ZFY 495bp
SRY 472bp	SRY 472bp
sY254 400bp (AZFc)	sY86 320bp (AZFa)
sY84 326bp (AZFa)	sY134 301bp (AZFb)
sY127 274bp (AZFb)	sY255 126bp (AZFc)



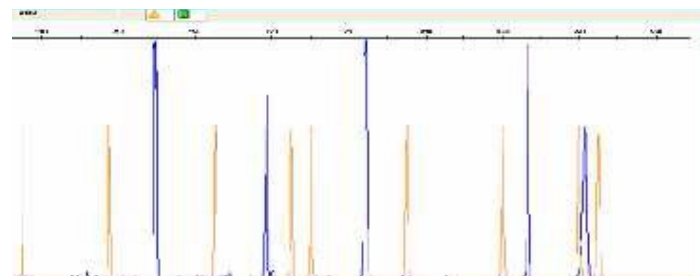
	STS	M-PCR Mix
AZFa	sY84	A
	sY86	B
	ZFX/ZFY SRY	
AZFb	sY127	A
	sY134	B
	ZFX/ZFY SRY	
AZFc	sY254	A
	sY255	B
	ZFX/ZFY SRY	

3) Simoni M. Int J Androl. 1999 22:292-9.

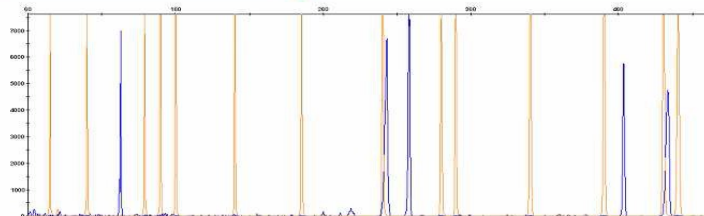
ANALYSIS OF RESULTS

Analysis performed on ABI 310 instrument:

MIX A



sY127 274 bp AZFb
sY84 326bp AZFa
sY254 379bp AZFc
SRY 472bp
ZFX/Y 495bp



sY255 126bp AZFc
sY134 301bp AZFb
sY86 320bp AZFa
SRY 472bp
ZFX/Y 495bp

References:

- 1) Kamp C et al. Hum. Mol. Genet. 2000 9:2563-72.
- 2) Repping S. et al. Am. J Hum Genet. 2002 71:906-22

Every STS recognized specific region of DNA and the amplification is related to the presence of a specific sequence of DNA on Y chromosome, whereas the absence means a deletion of the sequence.