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DETECTION OF AZF regions MICRODELETIONS of Y CHROMOSOME

AMPLI SET Y Chromosome Extension Cat. n. 1.502

Y Chromosome microdeletions of AZF regions (AZoospermia Factor) are observed in 10-15% of azoospermic men and 5-10% of oligospermic patients. 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).
BIRD-SET Y Chromosome Extension completes the BIRD-SET Y Chromosome since allows to confirm, using the PCR technique, microdeletions identified by the first step kit and to analyze the entire deleted region. BIRD-SET Y Chromosome extension kit is made of two mix PCR: 1) "Confirmation Mix" and 2)"Extension Definition Mix" of the microdeletions. The first group includes three mix-PCR, each allows the amplification of 1 STS inside one of the AZF regions (for AZFa: sY84: for AZFb: sY127; for AZFc: sY254). As a deletion is showed by the absence of PCR product, the mix PCR in the kit contains a primers pair specific for ZFX/ZFY genes that always produces an amplification product (internal PCR product). The purpose of this First Step PCR is the confirmation of the deletion detected with the BIRD-SET Y Cromosome UE.
The second group of mix PCR (Extension Mix PCR) allows the amplification of STS in the proximal and distal border of every AZF region. As the clinical significance of the deletion extensions (4,5), this second amplification, performed on the deleted samples obtained by the Confirmation PCR, allows to assess if a deletion is *complete* or *partial*. The choice of the markers is based on the directions of European Academy of Andrology Guide Lines (1) and on the data of recent literature.

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	
Confirmation PCR mix	-20°C
Extension PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
MALE GENOMIC DNA CONTROL	-20°C

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

ANALYSIS OF RESULTS

PCR products can be separated on agarose gel electrophoresis. The absence of PCR products of specific regions of Y chromosome shows the presence of a microdeletion of the sequence.

	Confirmation mix	Extension mix
AZFa	sY84 ZFX/ZFY	sY82 AZFa prox AZFa dist sY88
AZFb	sY127 ZFX/ZFY	sY105 sY114 sY143 sY1197 sY152
AZFc	sY254 ZFX/ZFY	sY143 sY158 sY160

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
3) Simoni M. Int J Androl. 1999 22:292-9.
4) Krausz C. et al. Hum. Reprod. 2000 15:1431-4.
5) Hopps C.V. et al. Hum. Reprod. 2003 18:1660-5
6) Kamp C et al. Mol. Hum. Reprod. 2001 7:987-94.