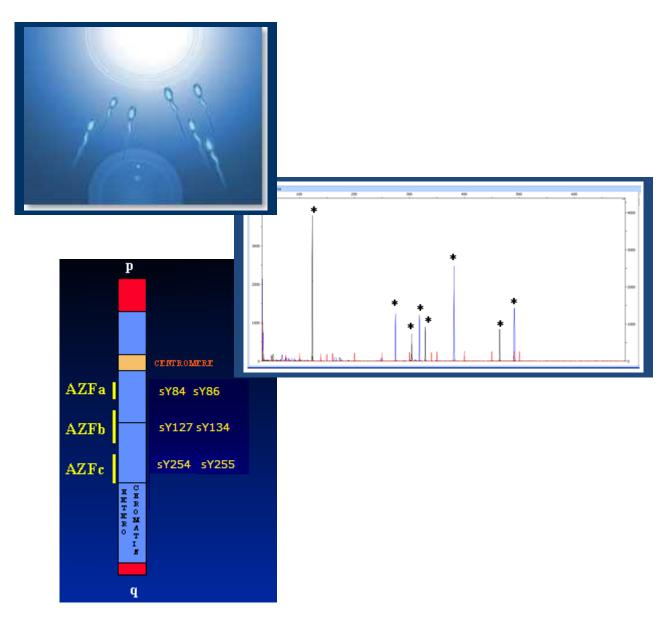


Complete System for detection of microdeletions of Y chromosome by single step PCR reaction in infertile men



cod. AZ.021FL



EXPERTEAM

via della Libertà 12 30175 Marghera Venice- Italy tel.: +39 041 5093101 fax: +39 041 5093102

(E IVD **Y chromosome microdeletions** are the second most frequent genetic cause of spermatogenic failure in unfertile men after Klinefelter syndrome. Incidence of these microdeletions in unfertile subjects related in literature is about 2-10%; it varies considerably depending on the selection criteria of the patients.

Portion of male-specific region in Y chromosome (MSY), where deletions map, has been subdivided in three regions called respectively **AZFa**, **AZFb**, **AZFc** (AZF = Azoospermia Factor). Microdeletions involve more frequently AZFc region (about 80%) respect to AZFb (about 9%), AZFbc (6%), AZFa (3%) and AFZabc (males XX: 3%). Different genes have been mapped in these regions, but their role in determining phenotype of deleted patients is not still clear.

Analysis of microdeletions of the long arm of Y chromosome, has assumed in the last years a great importance in diagnostic of unfertile subject and is currently effectuated in a great number of andrology centre and medicine of reproduction. Grow-up in the use of ICSI (Intracytoplasmatic Sperm Injection) has moreover contributed to the investigation of possible genetic cause, given risk of transmission of these alterations to the offspring. In fact patients with microdeletion of Y chromosome, are between the major candidates to ICSI, since are characterized by severe oligozoospermia or azospermia with possible presence of spermatozoa inside the testicle that can be recuperated through TESE (Testicular Sperm Extraction).

Kit content

Label	Contenut
AZO MASTER MIX -FL	Mix to amplify 6 STS and 2 control genes
ExperTaq polymerase	Taq DNA polimerasi



How does the kit work?

The Multiplex Oligo-azoospermia kit-FL, created following the guidelines published by the European Academy of Andrology and the EMQN (European Molecular Genetics Quality Network), utilized a panel of 6 STS (Sequence Tag Site) localized in the three AZF regions.

These STS are amplified by one PCR multiplex separated reaction and by capillary electrophoresis. The kit uses a three-dye fluorescent system and is validated for analysis on CEQ Genetic Analysis System (Beckman Coulter) and ABI Genetic Analysis System (Applied Biosystem). The ZFX/ZFY gene, chosen as internal PCR control, is present both in males and females DNA; it is useful for distinguishing negative results from failures of amplification reaction. The SRY gene has been included as a control for the testis-determining factor on the short arm of Y chromosome and for the presence of Y-specific sequence when ZFY gene is absent (male XX).

Starting samples: peripheral blood

DNA isolation method: QIAamp DNA blood mini kit, QIAcube, QIAsymphony (Qiagen), High Pure PCR template preparation kit (Roche).

DNA Sequencer: CEQ 8000/8800 Genetic Analysis System (Beckman Coulter); 310, 3100, 3130, 3730, 3500 Genetic Analyzers (Applied Biosystmes).

Procedure: according to the "EAA/EMQN Best Practice Guidelines for molecular diagnosis of Y-chromosomal microdeletions".

Why we should utilized this kit?

All the STS and control genes included in the kit are amplified together by single step **multiplex PCR reaction** and analyzed automatically by genetic analyser. This is a **cost effective** solution with **less hands-on time** required and without toxic EtBr stained agarose gels.

The **STS** utilized in this kit give the chance to make an accurate diagnosis of chromosome Y microdeletions because they detect almost all the relevant deletions from a clinic point of view and anyway over the 95% of deletions in the regions **AZFa**, **b**, **c** described in literature.