Y CHROMOSOME MICRODELETION

REAL TIME PCR KIT (8 REGIONS)

Cat. No: 15R-10-08

PRODUCT DESCRIPTION

Microdeletions of the Y chromosome are a recently discovered cause of spermatogenetic failure resulting in male infertility. In the last decade, many investigators have described the occurrence of microdeletions in infertile patients around the world. The molecular detection of deletions has become an important diagnostic test in male infertility studies. Four Azoospermia factors (AZFa, AZFb and AZFc) have been mapped to Yq11, of which AZFc is the most frequent region involved in deletions.

PRINCIPLE OF THE SYSTEM

During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

Each isolated DNA should be tested with each region real time pcr mastermixes. The kit provides reagents in a **"ready-to-use"** mastermix format which has been specifically adapted for 5' nuclease PCR using deletion analyses. The test system is designed for use with sequence specific primers and probe.

The fluorescence of AZF's analysis is FAM. Also each mastermix contains an internal control labelled with HEX/JOE dye.

SYSTEM CONTENTS

Reagents	10 rxns
SY14 (Control) Real Time PCR Mastermix	200 µl
ZFY (Control) Real Time PCR Mastermix	200 µl
SY84 (AZF-a) Real Time PCR Mastermix	200 µl
SY86 (AZF-a) Real Time PCR Mastermix	200 µl
SY127 (AZF-b) Real Time PCR Mastermix	200 µl
SY134 (AZF-b) Real Time PCR Mastermix	200 µl
SY254 (AZF-c/DAZ) Real Time PCR Mastermix	200 µl
SY255 (AZF-c/DAZ) Real Time PCR Mastermix	200 µl
Control DNA	90 µl

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>3X) should be avoided, as this may reduce the sensitivity of the assay.

DNA EXTRACTION

Blood samples should be collected in appropriate sterile EDTA tubes and can be stored at +4°C up to one month. For more than one month specimen should be stored at -20°C. It is advised to gently mix the tube (with EDTA) after collection of blood to avoid coagulation.

Our system optimized according to MN NucleoSpin ® Blood and Axygen Axyprep Genomic Blood DNA. It is advised to elute DNA with **150 µl elution buffer** for better results.

PROCEDURE

- Different tubes should be prepared for each region.
- Before starting work, mix the mastermixes gently by pipetting
- For each sample, pipet **20 µl mastermix*** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl (~10-100 ng) DNA** into each tube**.**
- Run with the programme shown below.

Master mixes include HotStart Taq DNA Polymerase.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	30 Cycles
60 °C	1 Min.	

Fluorescent dyes are FAM and HEX/JOE

If you use;

- ABI Prism® system, please choose **"none"** as passive reference and quencher.
- Mic qPCR Cycler, please adjust gain settings, **"Green Auto Gain"** to **20** and **"Yellow Auto Gain"** to **10.**

This system can use with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

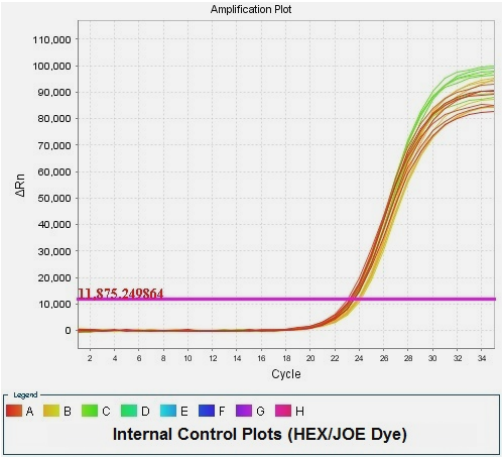
Roche LightCycler® 480 System

Rotor Gene Q

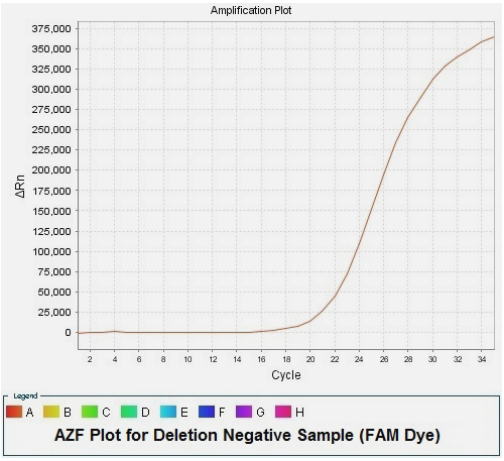
Mic qPCR Cycler

DATA ANALYSIS

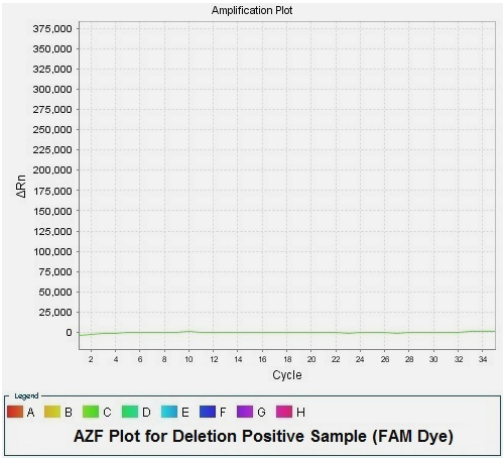
After the run is completed data are analysed using the software with HEX (JOE) and FAM dyes. The below results were studied with ABI7500.



Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX (JOE) dye. The CT value of internal controls should be **22 ≤ X ≤ 26**.



Amplification plots of AZF's can be analysed by FAM dye. The CT value should be between **18 ≤ CT ≤ 25**. These values are optimised according to the MN NucleoSpin ® Blood DNA Isolation Kit. CT values could be vary ±2/3 cycle according to the DNA isolation protocol.



TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

If plots start late,

Compare positive control and sample. If there is no problem in positive control,

- DNA quality is not good.
- The amount of DNA is not enough.

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.