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OCTO Health RT-PCR Y Chromosome Deletion Kit

Catalog #	Description
RT-YD-25	Real-Time PCR Y Chromosome Deletion Kit, 25 reactions
RT-YD-50	Real-Time PCR Y Chromosome Deletion Kit, 50 reactions

IVD

CE

Introduction

Y chromosome microdeletion is one of the most common genetic disorders associated with infertility in men. Y Chromosome Microdeletions are found in 10-15% of infertile men and are related to deletions in chromosome regions called the "Azoospermia Factor (AZF)," which are divided into three sections (AZFa, AZFb, AZFc). Partial or complete deletions in one or more of these regions result in very low or absent sperm production.

Diagnosis

In patients with azoospermia and/or oligospermia, a Y chromosome microdeletion test is conducted. Deletions in the AZF regions are detected using markers called STS (Sequence Tagged Sites) in the respective regions. Diagnostic methods that aim to identify STS regions through various molecular techniques are used to investigate the presence or absence of deletions

Technical Specifications

OCTO Health REAL TIME PCR Y Chromosome Deletion Kit is a multiplex PCR diagnostic kit that screens deletions in a total of nine regions in five tubes.

The Kit includes a total of 7 STS regions covering the AZF-a, AZF-b, and AZF-c regions, as well as the SRY region and the ZFX/Y region. The OCTO Health Real Time PCR Y Chromosome Deletion Kit operates using the Real Time PCR method. The SYBR Green-based Real Time PCR kit identifies 9 regions in a total of 5 separate tubes. In addition to the 5 tubes, it includes a separate control mix for validation, containing one STS region from AZF-a, AZF-b, and AZF-c.

Gene	Tube Number
SY84	1
SY134	
SY87	2
ZFX/Y	
SY254	3
SY127	
SY128	4
SRY	
SY255	5

	Mixes	25 Reactions	50 Reactions
1	Y-DEL Master Mix 1	500 µl	1000 µl
2	Y-DEL Master Mix 2	500 µl	1000 µl
3	Y-DEL Master Mix 3	500 µl	1000 µl
4	Y-DEL Master Mix 4	500 µl	1000 µl
5	Y-DEL Master Mix 5	500 µl	1000 µl

Required Equipment and Materials:

- Compatible RT-PCR Devices*

Biorad CFX96 and Opus, Qiagene Rotorgene, Quantstudio Real-Time PCR Systems, Roche LightCycler 480 & 96, Mic qPCR Cycler, Corbett Realtime PCR, Slan Realtime PCR, Molarray MA6000, Himedia Insta Q96, Q48 and Q48M

- DNA Isolation (Manual or automated system)
- Vortex and Centrifuge
- Pipette Set (0.5-10µl and 10-100µl)
- Sterile Pipette Tips
- Cold Block
- PCR tubes or plates compatible with the respective Real-Time PCR Device
- +4°C Refrigerator
- Sterile Gloves

** Please contact for other compatible devices*

Storage Conditions

- All components of the kit should be stored at -20°C until the expiration date.
- Kit components should undergo freeze-thaw cycles a maximum of 5 times. If more than 5 freeze-thaw cycles are anticipated, it is necessary to divide the kit components into smaller volumes for storage.

Experimental Preparation

Preparation of Materials:

- Materials stored at -20°C should be thawed on ice or cooling rack.
- Once thawed, liquid materials should be mixed well by pipetting.

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Preparation of Reactions:

Preparation of 5 tubes is required for each sample. Reaction volumes are specified in the table. The same tubes should be prepared for the NTC.

Content	Amount (µl)
PCR Master Mix Tube No 1-2-3-4-5	18
DNA Isolate / NTC	2
TOTAL	**20**

Real – Time PCR Protocol

Cycle	Temperature	Time	Fluorescent Dye
1	95 °C	300 sec.	
30	95 °C	30 sec.	
	60 °C	60 sec.	SYBR
Melt Analysis			
Initial Temp.	65 °C	**Ramp Rate**	0.3 (ºC /sec)
End Temp.	93 °C	**Hold**	2 sec.

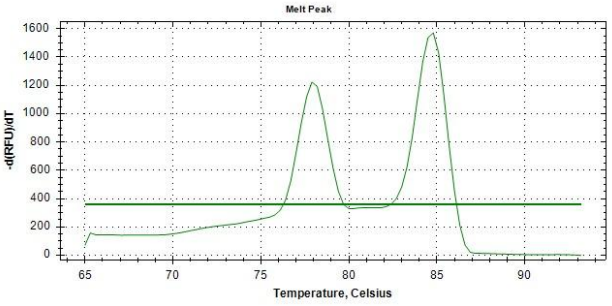
Expected Melt Temperatures

TUBE 1	Tm
SY134	79 °C
SY84	85 °C
TUBE 2	**Tm**
SY87	81 °C
ZFX/Y	84 °C
TUBE 3	**Tm**
SY127	81 °C
SY254	84 °C
TUBE 4	**Tm**
SY128	81 °C
SRY	85 °C
TUBE 5	**Tm**
SY255	83 °C

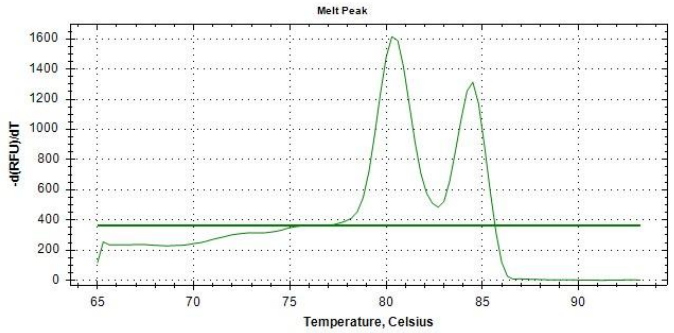
Data Analysis – Interpretation of Results

- After the Real-Time PCR stage, analysis begins by selecting the Melt Analysis option.
- Each tube must be analyzed separately.
- The analysis should be performed based on the presence or absence of peaks at the specified temperatures for each parameter.
- The expected peak temperatures and analysis evaluation for each parameter are shown in the table.

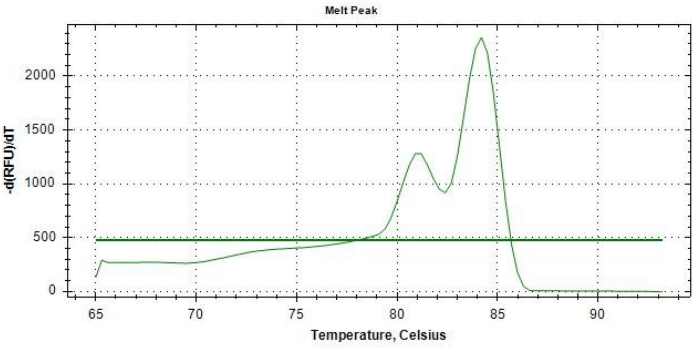
Sample Images



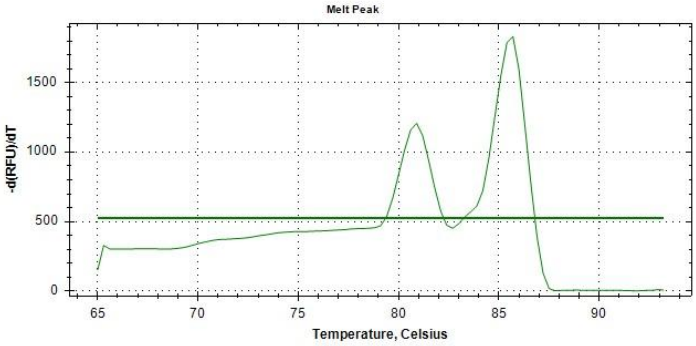
-Mix1- (SY134 & SY84)



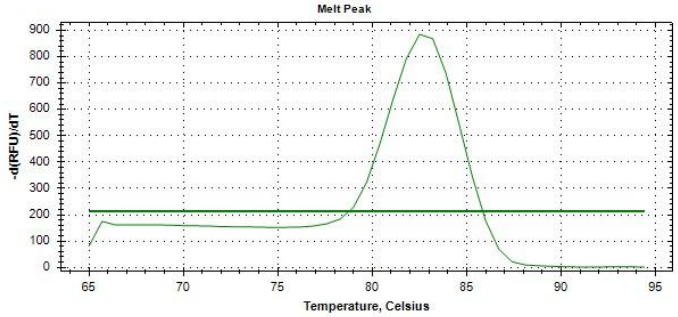
-Mix2- (SY87 & ZFX/Y)



-Mix3- (SY127 & SY254)



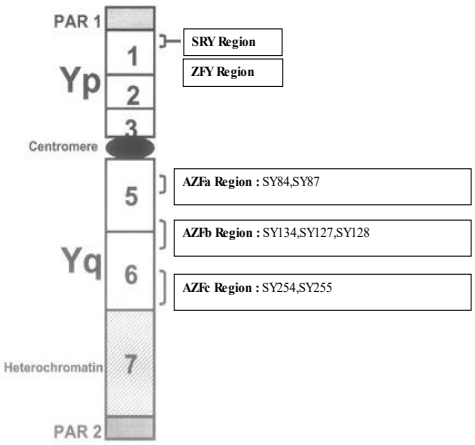
-Miks 4- (SY128 & SRY)



-Miks 5- (SY255)

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Troubleshooting

Problem: No Peaks Observed
Possible causes:

- The reaction mixes were not homogenous.
- An annealing temperature outside the protocol range was used.
- DNA isolation yielded too little or degraded template.
- Reagents (primers, probes, master mix) may be degraded or expired.

Suggested solutions:

- Vortex each mix thoroughly before dispensing to ensure uniformity.
- Verify thermocycler settings match the protocol's specified annealing temperature.
- Repeat DNA extraction, assess concentration and integrity (e.g. by NanoDrop or gel).
- Confirm all reagents are within their shelf-life; replace any that are expired.

Problem: Decrease in Fluorescence Intensity
Possible causes:

- Mixes were exposed to light or stored at room temperature for too long.
- Fluorescent probes or master mix have aged past their optimal storage conditions.

Suggested solutions:

- Keep all fluorescent reagents on ice, protected from light, and minimize time at ambient temperature.
- Store reagents exactly as directed (usually at –20 °C) and avoid freeze–thaw cycles.
- Replace any probes or mixes that have exceeded recommended storage duration.

Problem: Peak Observed Out of Expected Range
Possible causes:

- PCR cycling parameters (times, temperatures) deviate from protocol.
- Non-specific amplification or primer-dimer formation.
- Cross-contamination between samples or with positive controls.

Suggested solutions:

- Double-check and reprogram the thermocycler with the exact protocol settings; rerun the assay.
- Design or adjust primer concentrations to reduce non-specific products; include a melt-curve step to verify specificity.
- Perform all setup steps in a clean area, use fresh tips and tubes, and include no-template controls to monitor contamination.

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Warnings and Precautions

This test is for in vitro diagnostic purposes only and should be used strictly in accordance with the instructions provided by trained personnel. Clinical samples should be considered as materials potentially carrying an infection risk and should be prepared in a laminar flow cabinet.

• Do not use expired kit. • Avoid repeated freezing and thawing of reagents as it may reduce the sensitivity of the test. • Ensure that reagents are thawed before use, then gently mix the tubes and centrifuge briefly. • Prepare the reaction mixture quickly on ice or a cold block. • Establish two separate work areas, one for isolation and the other for PCR. • Avoid transferring pipettes, tubes, and other working materials between the work areas. • Always use filtered sterile pipette tips. • Use separate lab coats and gloves for each work area.

Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard **TS EN ISO 15223-1**


: **Number of Reactions**


: Expiration Date



: Lot Number



: Reference Number


: Temperature Limit


: Refer to the User Manual


: Manufacturer


: Keep Away From Direct Sunlight

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