

Y Chromosome Microdeletion (AZF) ToolSet™ for LightCycler™

Azoospermia Factor, Y chromosome microdeletions, Male Infertility

Detection of the STS AZFa : sy84 & sy86, AZFb : sy127 & sy134, AZFc : sy254 & sy255

Lyophilized ToolSet for PCR using the LightCycler™ 480 Instrument. Licensed by Roche Diagnostics GmbH

Order#: AZF - 16

1 ToolSet for 16 reactions

Store at 4°C, protected from light.
Exposure to light may especially damage
the OligoTool™ tubes (vials with red caps).

For use with LightCycler Fast Start DNA Master SYBR Green, 10 x conc. (Roche Cat.No.: 03003230001)

1. ToolSet contents

| Vial | Label | Content | Quantity |
|----------------------|--|--|---|
| 1-8, Red caps | OligoTools 1-8 | - lyophilized oligos for PCR - each contains a primer set for one STS | For 16 tests, each Dissolved: 25 µL, each |
| 9 and 10, Green Caps | Male and Female Normal Controls | - A : normal human male DNA, lyophilized (4x) - B : normal human female DNA, lyophilized (4x) | Dissolved: 20 µL, each |
| 11, Blue cap | Solvent | - to dissolve OligoTool / Control | 1000 µL |

Additional equipment and reagents required but not supplied :

LightCycler Fast Start DNA Master SYBR Green, 10 x conc. (Roche Cat.No.: 03003230001);

LightCycler 480 instrument, LightCycler microplates or microstrips, DNA extraction materials.

2. Introduction

2.1. Product overview

ToolSet description

The **AZF ToolSet** is specifically designed for detection of AZF **microdeletions in the human Y chromosome** by LightCycler PCR with Melting Curve Analysis. The AZF ToolSet contains 8 singleplex reactions for the STS (Sequence Tagged Sites) recommended for AZF testing : sy84 and sy86 for the AZFa region, sy127 and sy134 for the AZFb region, sy254 and sy255 for the AZFc region, SRY to control for presence of the Y chromosome and ZFY/ZFX as positive amplification control (Simoni 2004).

Control material

A : normal human **male** DNA, lyophilized **B** : normal human **female** DNA, lyophilized
With Control A, all reactions will yield a product.
With Control B, only the ZFY/ZFX reaction will yield a product.

Storage of ToolSet

Store at +4°C when lyophilized, protected from light. The unopened lyophilized ToolSet is stable at +4°C for 12 months from date of manufacture if protected from light. When dissolved store at +4°C for a maximum of 3 days, or at -20°C for longer periods (months), protected from light. Avoid freezing and thawing > 3x.

3. Preparation for LightCycler PCR

**Toolset
preparation**

1. Before opening tubes, centrifuge them quickly.
2. **Dissolve** the content of the **OligoTool** tubes (Red Caps) with **25 µl of Solvent**.
3. **Dissolve** the content of the **Control** tubes (Green Caps) with **20 µl of Solvent**.
4. Recap tubes, vortex gently, centrifuge shortly and proceed to Reaction Mix preparation.

**Reaction Mix
Preparation**

Prepare LightCycler Fast Start DNA Master SYBR Green (Roche Cat.No.: 03003230001) as indicated in the Roche pack insert and place in a cooled metal block (+ 4 to + 6 °C).

For each DNA sample to be tested, 8 reactions will be set up.

For **ONE** DNA sample, prepare the **Mix A** as shown in the following table, mix gently, centrifuge briefly and keep cooled until the next step :

| Reagent | µL |
|---------------------------------------|-----------|
| Solvent (Blue cap tube) | 18 |
| MgCl ₂ 25mM | 12 |
| Fast Start DNA Master SYBR Green, 10x | 10 |
| Total Mix A | 40 |

For **ONE** DNA sample, prepare **8 individual primer Mixes** as shown in the following table, mix gently, centrifuge briefly and keep cooled until the next step :

| Reagent | µL |
|--|------------|
| Mix A | 4.4 |
| Individual Dissolved OligoTool | 1.1 |
| Total Individual Primer Mixes 1 – 8, each | 5.5 |

**DNA sample
Dilution**

Dilute your DNA sample(s) as shown in the following table :

| Reagent | µL |
|--------------------------|-----------|
| DNA sample | 18 |
| Solvent (Blue cap tube) | 27 |
| Total Diluted DNA | 45 |

**Reaction
Setup**

For each sample to be tested, use 8 (eight) microplate wells. Into each microplate well, dispense 5 ul of one of the 8 individual primer Mixes.

We suggest that you use the order of the STS positions in the Y chromosome :

| Microplate well # | OligoTool |
|-------------------|-----------|
| A | sy84 |
| B | sy86 |
| C | sy127 |
| D | sy134 |
| E | sy254 |
| F | sy255 |
| G | SRY |
| H | ZFY/ZFX |

Centrifuge plate / strip briefly, then and add 5 ul of your diluted DNA sample to each well. Cap or seal Microplate / Microstrip wells, centrifuge and run the LightCycler program.

More Samples ?

For more samples to be tested, multiply volumes for Mix A and the 8 individual primer Mixes appropriately.

Layout

We suggest that you use the following microplate / microstrip layout as in the example below which assumes three Proband DNAs :

Arrange the 8 individual primer Mixes horizontally :

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---------|---------|---------|---------|---------|---------|
| A | sy84 | sy84 | sy84 | sy84 | sy84 | sy84 |
| B | sy86 | sy86 | sy86 | sy86 | sy86 | sy86 |
| C | sy127 | sy127 | sy127 | sy127 | sy127 | sy127 |
| D | sy134 | sy134 | sy134 | sy134 | sy134 | sy134 |
| E | sy254 | sy254 | sy254 | sy254 | sy254 | sy254 |
| F | sy255 | sy255 | sy255 | sy255 | sy255 | sy255 |
| G | SRY | SRY | SRY | SRY | SRY | SRY |
| H | ZFY/ZFX | ZFY/ZFX | ZFY/ZFX | ZFY/ZFX | ZFY/ZFX | ZFY/ZFX |

Arrange Controls and Proband DNAs vertically :

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---------|------|--------|--------|--------|--------|
| A | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |
| B | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |
| C | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |
| D | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |
| E | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |
| F | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |
| G | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |
| H | Neg Con | Male | Female | Prob 1 | Prob 2 | Prob 3 |

Male Control

Always run a normal male DNA control with the samples. Use the dissolved **Normal Male Control** DNA (Green Cap).

Female Control

Always run a normal female DNA control with the samples. Use the dissolved **Normal Female Control** DNA (Green Cap).

Negative control

To prepare a negative control, replace the template DNA with Solvent (Blue Cap).

Extraction of genomic DNA

You can use different Kits for DNA isolation, either with a manual method or with an automated system. The elution buffers should be low-salt or salt-free.

Application

The **AZF ToolSet™ for LightCycler™** has been designed for detecting the STS recommended for AZF testing : sy84 and sy86 (AZFa), sy127 and sy134 (AZFb), sy254 and sy255 (AZFc); SRY and ZFY/ZFX as positive controls. STS are amplified in parallel singleplex reactions and detected by closed – tube melting curve analysis.

Note : This ToolSet was developed for use in life science research only.

4. LightCycler 480 Settings and Experimental Protocol

For use with LC 480 Program Version 1.5 series. Detection : Dynamic SybrGreen I / HRM 465 – 510 nm.

Fast Start Enzyme Activation and DNA Denaturation

| Cycle Program Data | Value |
|---|------------------|
| Cycles | 1 |
| Analysis Mode | None |
| Temperature Targets | Segment 1 |
| Target Temperature (°C) | 95 |
| Incubation time (s) Hold | 600 |
| Temperature Transition Rate (°C/s) Ramp Rate | 4.4 |
| Acquisitions (per °C) | --- |
| Secondary Target Temperature (°C) | 0 |
| Step Size (°C) | 0 |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

Amplification

| Cycle Program Data | Value | | |
|--|------------------|------------------|------------------|
| Cycles | 30 | | |
| Analysis Mode | Quantification | | |
| Temperature Targets | Segment 1 | Segment 2 | Segment 2 |
| Target Temperature (°C) | 95 | 60 | 72 |
| Incubation time (s) Hold | 10 | 10 | 1 |
| Temperature Transition Rate (°/s) Ramp Rate | 4.4 | 2.2 | 4.4 |
| Acquisitions (per °C) | --- | --- | --- |
| Secondary Target Temperature (°C) | 0 | 0 | 0 |
| Step Size (°C) | 0 | 0 | 0 |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | None | Single |

Melting Curve Analysis

| Cycle Program Data | Value | | |
|--|------------------|------------------|------------------|
| Cycles | 1 | | |
| Analysis Mode | Melting Curves | | |
| Temperature Targets | Segment 1 | Segment 2 | Segment 3 |
| Target Temperature (°C) | 95 | 40 | 99 |
| Incubation time (s) Hold | 30 | 30 | --- |
| Temperature Transition Rate (°/s) Ramp Rate | 4.4 | 2.2 | 0.1 |
| Acquisitions (per °C) | --- | --- | 6 |
| Secondary Target Temperature (°C) | 0 | 0 | 0 |
| Step Size (°C) | 0 | 0 | 0 |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | None | Continuous |

Cooling

| Cycle Program Data | Value |
|--|------------------|
| Cycles | 1 |
| Analysis Mode | None |
| Temperature Targets | Segment 1 |
| Target Temperature (°C) | 40 |
| Incubation time (s) Hold | 30 |
| Temperature Transition Rate (°/s) Ramp Rate | 2.2 |
| Secondary Target Temperature (°C) | 0 |
| Step Size (°C) | 0 |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

5. Typical results

Introduction

Use the Melting Curve program to genotype the human genomic DNA research samples.

The following **table** summarizes the **T_m** values for each STS / OligoTool.

Note : The values for the respective melting temperatures may vary for +/- 2.5 °C between different experiments.

| OligoTool | No DNA T _m (°C) | Normal Female T _m (°C) | Normal Male T _m (°C) |
|-----------|----------------------------|-----------------------------------|---------------------------------|
| sy84 | No peak | No peak | 87 |
| sy86 | No peak | No peak | 87 |
| sy127 | No peak | No peak | 83 |
| sy134 | No peak | No peak | 79 |
| sy254 | No peak | No peak | 82 |
| sy255 | No peak | No peak | 86 |
| SRY | No peak | No peak | 88 |
| ZFY / ZFX | No peak | 86 | 86 |

Interpretation

**Male proband DNA must show peaks for the controls SRY and ZFY / ZFX.
Absence of peaks for other STS indicate Y chromosome microdeletions :**

| OligoTool | Male proband DNA T _m (°C) | Y chromosome microdeletion |
|-----------|--------------------------------------|----------------------------|
| sy84 | No peak → | AZFa |
| sy86 | No peak → | AZFa |
| sy127 | No peak → | AZFb |
| sy134 | No peak → | AZFb |
| sy254 | No peak → | AZFc |
| sy255 | No peak → | AZFc |

The AZF ToolSet™ has been developed for and validated with the LightCycler™ and its original accessory materials and reagents. Ratiogen has not validated the ToolSet with other instruments, accessories or reagents.

Examples of Melting Curves : see next page

7. Notices to Purchaser

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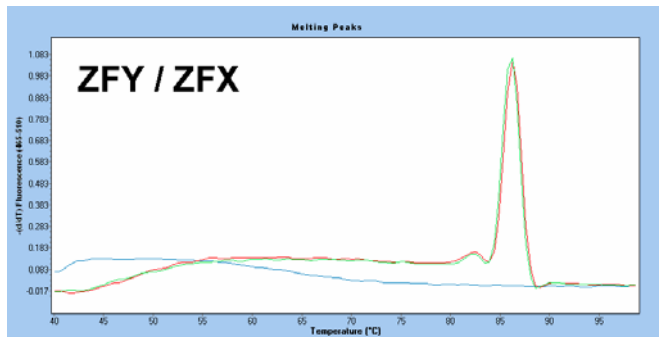
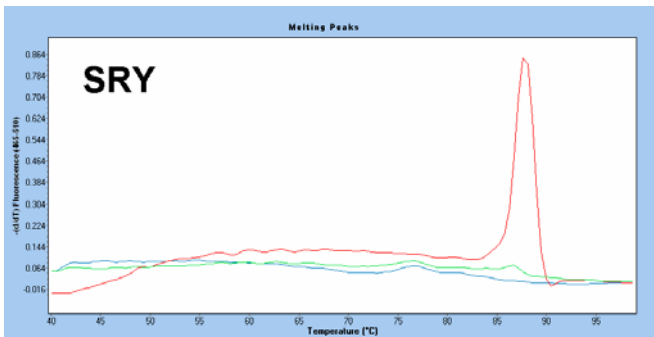
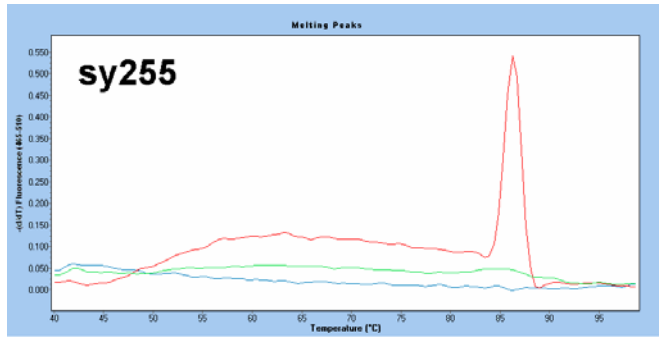
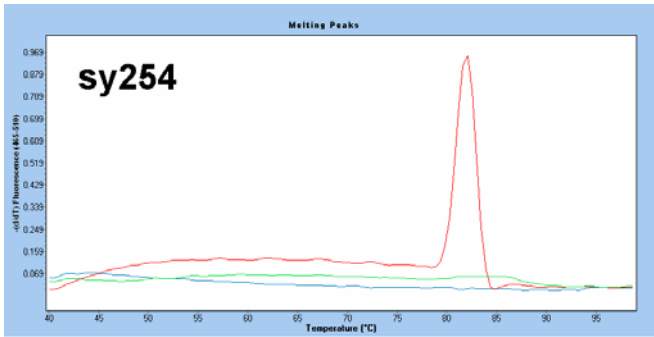
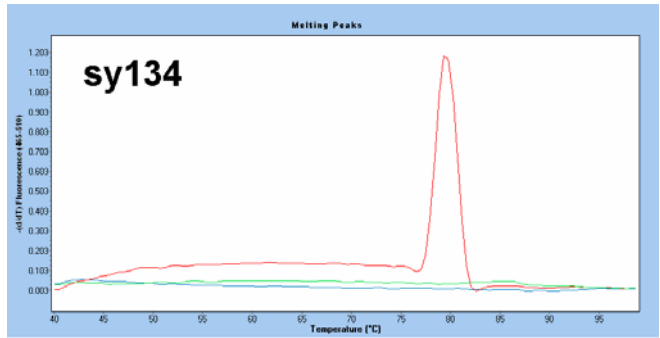
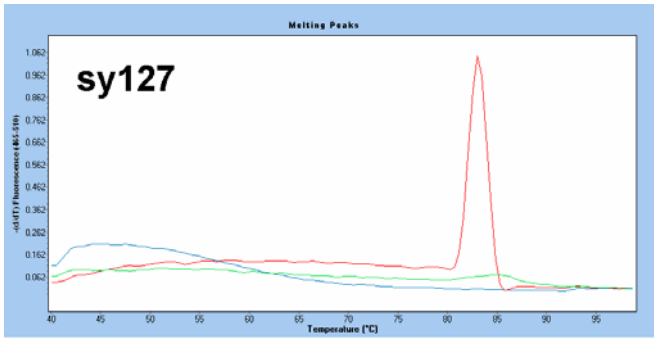
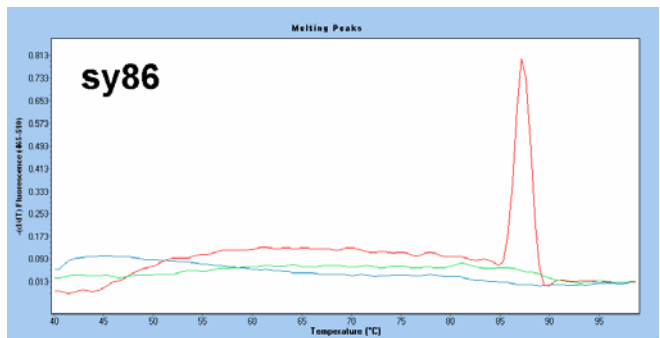
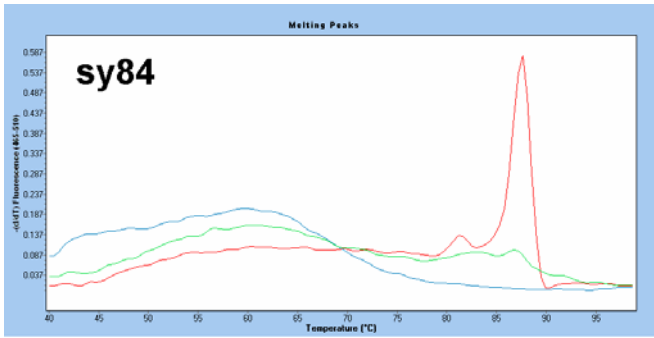
How to contact ratiogen

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Protocol uses Fast Start DNA Master SYBR Green !

Y chromosome microdeletion (AZF) Analysis by LightCycler : Melting Curve Examples



No DNA

Normal Male DNA

Normal Female DNA