

Devyser AZF RUO Art. No.: 8-A019-RUO For Research Use Only

Handbook

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1. Introduction to Devyser AZF

Intended use	For research use only. Not for use in diagnostic procedures.
Included in the kit	The Devyser AZF kit contains ready-to-use reagents for PCR amplification of genetic markers.
Test procedure	<u>DNA extraction</u> – The Devyser AZF kit has been optimised using QIAamp DNA Blood Mini Kit (Qiagen, cat#51104) for extraction of DNA from human whole blood. <u>Amplification</u> – The Devyser AZF kit has been optimised using ABI GeneAmp [®] Systems 9600/9700/2720, Eppendorf Master- cycler and MJ Research/Bio-Rad PTC200 with 96-well alpha unit. <u>Detection</u> <u>Applied Biosystems</u> Genetic Analyzers (ABI PRISM [®] 310, 3100, 3130, 3500, 3730) that support detection of 6-FAM. <u>MegaBACE</u> systems that that support detection of 6-FAM

Principle of the Procedure Genetic testing using the Devyser AZF kit relies on PCR amplification of sequence-tagged sites (STS) of the AZFa, AZFb and AZFc regions of the Y-chromosome. Succesful amplification of a STS marker indicates prescence whereas absence of PCR amplification is indicative of deletion. Primers for a total of 14 markers are combined into one multiplex PCR reaction. All of the loci recommended by the European Academy of Andrology (EAA) and the European Quality Monitoring Network Group (EMQN) are included in the kit.

The ZFX/ZFY genes are used as internal control of the PCR amplification, as these primers amplify unique fragments both on the Y-chromosome (ZFY) and on the X-chromosome (ZFX). The SRY gene is included in the analysis as control for the testis determining factor on the short arm of Y chromosome and for the presence of Y-specific sequences in the case that the ZFY gene is absent

2. Warnings and Precautions

- **A.** Devyser AZF has been optimised using a total PCR reaction volume of 25 μL. Changing the reaction volume will compromise the kit performance.
- **B.** Avoid microbial contamination of reagents when removing aliquots from reagent vials. The use of sterile disposable aerosol barrier pipette tips is recommended.
- C. Do not pool reagents from different lots or from different vials of the same lot.
- **D.** Do not use a kit after its expiry date.
- **E.** Do not use opened or damaged kit reagent vials.
- F. Work flow in the laboratory should proceed in a uni-directional manner, beginning in the reagent preparation area and moving to the DNA extraction area and then to the amplification area and finally to the detection area. Pre-amplification activities should begin with reagent preparation and proceed to DNA extraction. Reagent preparation activities and DNA extraction activities should be performed in separate areas. Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas. Gloves should be worn in each area and should be changed before leaving that area. Equipment and supplies used for reagent preparation should not be used for DNA extraction activities or for pipetting or processing amplified DNA or other sources of target DNA. Amplification and detection supplies and equipment should remain in the amplification and detection area at all times.
- **G.** Handling of kit components and samples, their use, storage and disposal should be in accordance with the procedures defined by national biohazard safety guidelines or regulations.
- H. Wear powder free disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

3. Symbols used on Labels



Lot or batch number



Expiry date



Manufacturer



Number of tests



Store below temperature shown

4. Required Material

4.1 Included in the Devyser AZF kit (#8-A019-RUO)

Configuration The Devyser AZF kit contains reagents for analysis of maximum 25 samples.

Components

Cap Colour	Tube Colour	Label	Art.Nr.	Kit Content
Orange	Clear	PCR Activator	4-A018	1x25 Tests
White	Amber	Devyser AZF Mix	4-A020	1x25 Tests

4.2 Required but Not Provided

Reagent Prepara- tion	 Consumables for the Thermal Cycler. Micropipette/dispenser with aerosol barrier tips or displacement tips (500 μL). Disposable protective gloves (powder free).
DNA Extraction	 Reagents and equipment according to manufactur- er instructions for use. Micropipette/multipipette with aerosol barrier tips.
Amplification	 Thermal Cycler: ABI GeneAmp[®] PCR System 9600/9700/2720 or Eppendorf Mastercycler or MJ Research/Bio-Rad PTC200 with 96-well alpha unit. Micropipette/dispenser with aerosol barrier tips or displacement tips (5, 20 µL).
Detection	 Applied Biosystems Genetic Analyzer (ABI PRISM[®] 310, 3100, 3130, 3500, 3730). Performance optimized polymers: POP-4, POP-6 or POP-7 <u>Devyser, Size Standard</u> <i>Devyser Dye-Set DEV-5</i>: 560 SIZER ORANGE (Devyser cat.# 8-A402)
	• <u>ABI Prism Size Standard Gene-Scan-500:</u> <i>Dye Set D:</i> Gene-Scan-500 ROX Size Standard (ABI cat.#401734/#4310366). <i>Dye Set G5:</i> Gene-Scan-500 LIZ Size Standard (ABI cat.#4322682).
	 Hi-Di Formamide, Genetic Analysis Grade (ABI cat.#4311320). 1x Genetic Analyzer Buffer Micropipette/multipipette/dispenser with aerosol barrier tips or displacement tips (1,5 uL, 15 uL).
	Dye Set Calibration: <u>ABI 3100, 3130, 3730:</u> Use DEV-5 Dye Set MultiCap kit (Devyser cat# 8-A401) in the "Any5Dye" dye set. <u>ABI 3500:</u> Use DEV-5 Dye Set MultiCap kit (Devyser cat# 8-A401) <u>ABI 310 Matrix file generation:</u> Here DEV 5 Dye Set Single Care kit (Devyser cat# 2, 4400)

Use: DEV-5 Dye Set SingleCap kit (Devyser cat# 8-A400). Run with module file "GS STR POP4 (1 mL) G5.md5"
 Detection
 Dye Set Calibration (contd.): ABI 310/3100/3130/3500/3730: Dye Set D: DS-30 or DS-31 Dye Set G5: DS-33

> MegaBACE: 6-FAM dye for AZF detection ROX for size standard detection

5. Storage and Handling Requirements

Α.	Store all components below -18°C.
В.	Reaction Master Mix (prepared by addition of Devyser AZF Mix to PCR Activator) may be stored at $+2$ to $+8^{\circ}$ C for at least 7 days and at below -18 C for at least 90 days. Avoid repeated freeze-thawing.
С.	Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
D.	Do not mix reagents from different kit lot numbers.

6. Sample Requirements

Clinical Samples	The Devyser AZF kit is for use with human genomic DNA.
Procedure & Storage	According to manufacturer instructions for use.
Controls	It is recommended that suitable controls such as normal male DNA, normal female DNA and negative control (no DNA) are included in each run (see section 8).

7. Instructions for Use

Run Sizes Each Devyser AZF kit (Art # 8-A019-RUO) contains reagents for 25 samples.

It is recommended that the activated reaction mix is dispensed into appropriate PCR reaction vials after preparation. Before dispensing ensure that the activated reaction mix is properly mixed (see section 7.1). Dispense in 20 μ L aliquots and store at below – 18 °C.

To avoid contamination always use un-opened vials. Any reagents left in opened vials should be discarded.

7.1 Workflow Devyser AZF kit (Art # 8-A019-RUO)

The Reaction Master Mixes should be prepared before preparing the samples, if the complete process is performed in one day. Only if the samples are prepared the day before amplification or earlier, the opposite order is advisable.

The Reaction Master Mix is prepared by adding the Devyser AZF Mix to the PCR Activator.

Devyser AZF has been optimised using a total PCR reaction volume of 25 $\mu L.$ Changing the reaction volume will compromise the kit performance.

Needed reagents from the kit are PCR Activator and Devyser AZF Mix.

Reagent Preparation Area:

1. Centrifuge each tube briefly to collect the content. Do not vortex the tubes at this step!

2. Carefully add 500 μL Devyser AZF Mix to the PCR Activator tube.

NOTE! >>>> 3. <u>Mix manually by pipetting</u> 300-500 µL several times from the bottom of the tube.

4. Vortex the Reaction Master Mix vial and centrifuge briefly to collect the content.

5. Add 20 μ L of Reaction Master Mix to separate PCR reaction tubes (8-strip of tubes or micro well plate).

6. Cap the reaction tubes and centrifuge briefly to collect the contents.

The Reaction Master Mix is stable at +2-8°C for at least 7 days and at below -18 C for at least 90 days. Avoid repeated freeze-thawing.

7.2. Sample Preparation And PCR amplification

DNA Ex-	According to manufacturer's instructions for use.
	DNA concentration and DNA purity are important factors for suc- cessful testing using the Devyser AZF kit. DNA should be free from contaminating protein and salts. Poor quality DNA may re- sult in increased background or amplification failure. Addition of too much or too little DNA to the PCR reaction can cause amplifi- cation failure.
	For recommended PCR conditions and analysis settings (see below), results are consistently obtained at DNA concentrations between 100 and 200 ng genomic DNA/PCR.
Addition of Sample	Samples should be added in a dedicated area separated from rea- gent preparation, amplification and detection areas.
	1. Add 5 μL of clinical sample (20-40 ng genomic DNA/ μL) to dedicated PCR reaction tubes containing Reaction Master Mix (from step 7.1)
	2. Cap the tubes and centrifuge briefly to collect the content.
Amplification	Turn on the Thermal Cycler at least 30 minutes prior to amplification.
	 For Eppendorf Mastercycler use "CNTRL TUBE" mode For GeneAmp[®] System 9700 set "ramp speed" to "9600 mode". For MJ Research/Bio-Rad PTC200 with 96-well alpha unit, use "Calculated vial temperature" and "MAX" ramping mode.
	Amplification Area: Program the Thermal Cycler for amplification according to the following Thermo Profile (consult the User's Manual for additional information on programming and operation of the thermal cycler):
	95°C 15 min 94°C 30 sec; 62°C 90 sec; 72°C 90 sec for 21 cycles 72°C 30 min 4°C FOREVER

7.2. Sample Preparation And PCR amplification (contd.)





1. Set reaction volume to 25 μ L.

2. Start the amplification (duration approximately 2hrs).

3. Following amplification, remove the tubes containing completed PCR amplification reaction from the thermal cycler and place into a suitable holder. Centrifuge briefly to collect the content. Remove the caps carefully to avoid aerosol contamination. Do not bring amplified material into the pre-amplification areas. Amplified material should be restricted to amplification and detection areas.

7.3. Detection

Sample preparat- ion	Refer to the respective ABI PRISM [®] Genetic Analyzers User Manual for instructions on maintenance and handling. Prior to running the Devyser AZF kit, the instrument must be spectrally calibrated to support detection of the FAM dye with the polymer used.		
	Sample Preparation for ABI 310/ 3100/ 3130/ 3500/ 3730		
	1. Prepare a loading cocktail by combining and mixing 3 μL of the size standard with 100 μL Hi-Di Formamide (sufficient mix for 6 wells/tubes).		
	2. Vortex for 15 seconds.		
	3 . Dispense 15 μ L of the loading cocktail into the required number of wells of a microwell plate or into individual tubes (ABI310) to be placed on the Genetic Analyzer.		
	4. Add 1,5 μ L of the sample PCR product to the corresponding well/tube containing loading cocktail.		
	5. Seal the plate/tubes.		
Instrument Preparation	Create a sample sheet using the data collection software with the following settings: Sample ID. Size standard Dye Set: DEV-5/Any5, D or G5.		
	» Recommended run Medules See below for different polymers		

» Recomended run Module: See below for different polymers and instruments.

Run Modules

ABI 310

Run Parameters	POP-4
Capillary length	47 cm
Run temperature	60
Injection voltage	8
Injection time	2
Run voltage	15
Run time	30 min

ABI 3100/3130

Run Parameters	POP-4	POP-7
Capillary length	36 cm	36 cm
Run temperature	60	60
Injection voltage	1,5	1,5
Injection time	10	10
Run voltage	15	15
Run time	1800 s	1800 s

ABI 3500

Run Parameters	POP-7	
Capillary length	50 cm	
Run temperature	60 °C	
Injection voltage	1,6 kV	
Injection time	8 s	
Run voltage	19,5 kV	
Run time	1500 s	

The amount of PCR product injected into the capillaries can be adjusted by increasing/decreasing the injection voltage and/or injection time.

8. Results and Analysis

Principle of Detection

Chromosome-specific markers known as sequence tagged sites (STS) are amplified by PCR. By the use of fluorescently labeled primers the visualization and identification of the PCR products are performed using a Genetic Analyzer with associated software

Data Analysis

The marker peaks in the electrophoretogram should be identified according to the specific marker sizes as presented in the marker overview (page 16).

Presence of a STS marker fragment is consistent with a non-deleted STS marker.

Absence of a STS marker is consistent with a deleted STS marker.

Flourescence Criteria

The acceptable range for marker peak height is above 200 relative fluorescent units. If peaks are saturated it is recommended that samples are re-injected with reduced injection time and/or injection voltage.

Determine that the control samples are approved prior to analyzing the clinical samples.

Negative Control

No specific peaks should be present within the range 100-500 bp.

Normal Male Control

The number and size of the peaks are indicated in the marker overview (page 16). All peaks according to the marker overview must be present. See figure 1.

Normal Female Control

Only one peak generated from marker ZFX should be present. See figure 2.

Unknown Samples

Determine the presence or absence of marker specific peaks. All deletions should be contiguous. If multiple marker peaks are missing, and those peaks do not map to adjacent regions of the Y chromosome they represent dropout peaks and are not deletions.

Markers sY254 and sY255

STS markers sY254 and sY255 are present in at least 4 copies each on the Ychromosome. In male samples with intact STS loci, both markers are expected to generate peak heights exceeding the peak height of all other markers detected.

Marker Overview Devyser AZF

STS Marker	Locus	Map position (page 17)	Marker size * (±2,5 bp)
sY255**	DAZ1-4	13	123 bp
sY131	DYS222	10	169 bp
sY90	DYS278	8	175 bp
sY81	DYS271	3	208 bp
sY625	G65849	5	254 bp
sY127**	DYS218	9	273 bp
sY157	DYS240	14	290 bp
sY134**	DYS224	11	304 bp
sY86**	DYS148	4	318 bp
sY84**	DYS273	6	329 bp
sY254**	DAZ1-4	12	376 bp
M259	DDX3Y	7	396 bp
ZFY/ZFX	ZFY / ZFX	2	432/433 bp
sY14 (SRY)	SRY	1	464 bp

*Based on observed marker sizes using ABI3130 and POP7. ** Basic set of STS primers according to EAA/EMQN best practice guidelines (Ref1)

Schematic overview of the marker positions on the Y-chromosome.

Мар	STS	Locus	Region	
1	sY14	SRY	V-11 D	
2	ZFY	ZFY	Yp11.3	
3	sY81	DYS271	Yq11.21	
4	sY86	DYS148		
5	sY625	G65849	475-	
6	sY84	DYS273	Ага	
7	M259	DDX3Y		
8	sY90	DYS278	Yq11.221	
9	sY127	DYS218		
10	sY131	DYS222	AZFb	
11	sY134	DYS224		
12	sY254	DAZ1-4		
13	sY255	DAZ1-4	AZFc	
14	sY157	DYS240		

 $\underline{\mbox{Figure 1.}}$ Typical results obtained from a normal male sample. All STS markers are present.



<u>Figure 2.</u> Typical results for obtained from a normal female sample. All Y-chromosomal STS markers are absent and only the X-chromosomal ZFX control locus is detected.

120	160	200	240	280	320	360	400	440
1200								
1000								
800								
600								
400 -								
200 -								
0								(mmad

<u>Figure 3.</u> Typical results obtained from an abnormal male sample. Markers sY255, sY157 and sY254 are absent which is consistent with deletion of the AZFc region.



In the case of absence of a single STS marker it is recommended that follow-up studies are performed to identify the reason.

If electrophoretograms are of poor quality (peak smears/aberrant sizing or electrophoretic spikes) it is recommended that the sample is re-injected using fresh reagents for electrophoresis (Polymer, buffer etc.).

If a marker displays inconclusive results or a single marker is not detected a number of reasons are possible:

- » Primer site deletion
- » Contaminating DNA: second genotype, PCR amplicons.
- » Primer site polymorphism/mutation.
- » DNA concentration used is too high or too low.
- » DNA used in PCR is degraded.
- » Electrophoretic spike

9. References

1. Simoni M, Bakker E, Krausz C. International Journal of Andrology, 27:240-249 (2004). EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004.

10. Notice to Purchaser

For research use only. Not for use in diagnostic procedures.

Purchase of this product does not provide a license to perform PCR under patents owned by any third party.

11. Contact Information

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