RepoDx®

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Y-chromosomal microdeletions Deletion Detection Kit
Package insert
For In Vitro Diagnostic Use Only

REF

For use with *ABI 7500 Real-Time PCR System*

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1. Intended Use

The kit can be used for the diagnosis of Y-chromosomal microdeletions of the AZFa (sY84 and sY86), AZFb (sY127 and sY134), and AZFc (sY254 and sY255) region on azoospermic and oligozoospermic men. It is only used for clinical auxiliary diagnosis. In clinical application, the clinician should also consider the actual situation of each case, and the test conclusion of this kit cannot be used as the only basis for clinical diagnosis.

Indications: The detection before intracytoplasmic sperm injection (ICSI) assisted reproductive therapy technology, genetic testing of male patients with oligospermia and azoospermia, patients with normal sperm density but unknown cause of infertility, male infertility patients with cryptorchidism and varicocele, the wife of the patient had an unexplained habitual abortion, sperm library screening, and et al.

2. Principle

The kit is based on the principle of fluorescence PCR method and Taqman fluorescence probe technique, PCR amplification of target DNA and detection of cleaved multi-labeled oligonucleotide detection probe specific to the target DNA. The Master Mix reagent contains primers specific to 6 sequence-tagged site (STS) in Y-chromosomal microdeletions of the AZFa (sY84 and sY86), AZFb (sY127 and sY134), and AZFc (sY254 and sY255). The detection of amplified DNA is performed using target specific multi-labeled oligonucleotide probes that permit independent identification of ZFX gene (located on X and Y chromosomes) and SRY gene (testicular determinant, located on Y chromosomes) amplicon.

3. Kit Contents

Label	Main Compositions	Number	Volume/ tube
PCR reaction mix 1	SY84, SY127, SY255, ZFX, and SRY primers and probes; Taq polymerase; UDG; dNTPs	1	1.44ml
PCR reaction mix 2	SY86, SY134, SY254, ZFX, and SRY primers and probes; Taq polymerase; UDG; dNTPs	1	1.44ml
Quality control 1	Normal male genome DNA	1	0.20ml
Quality control 2	Normal female genome DNA	1	0.20ml
Package insert	//	1	1 sheet

Notes: Different batches of components cannot be mixed
This kit does not contain sampling and extraction components

4. Reagent Storage and Handling

- This kit must be stored below -18 °C and protected from light.
- Repeated freeze-thaw cycles should be less than 6 times.
- The kit can be stored for 12 months before opening. Once opened, any unused portion can be stored up to 1months.

5. Specimens Applied to Test

5.1 Human EDTA anticoagulant whole blood, human sodium citrate anticoagulant whole blood, or human oral exfoliated cell.

5.2 Sample collection was carried out according to *the clinical testing requirements of the Ministry of Health*.

5.2.1 Oral exfoliated cells obtaining: gargle 2-3 times with water, scrape 5-6 times with a sterile cotton swab on both sides of the inner wall of the mouth, put the swab into a 1.5 ml centrifugal tube containing 1 mL saline, wash the cotton swab with full oscillation, then squeeze the swab against the pipe wall to dry and discard.

5.2.2 Blood sample: the venous blood of the patient was injected into a sterile blood collection tube or a centrifuge tube. EDTA or sodium citrate can be used as anticoagulant, avoiding the use of heparin anticoagulants.

5.3 PCR interfering substances
Hemoglobin and mucin.

5.4 Storage and transportation of samples:
Samples can be used immediately
or stored at 2-8 ° C (no more than 7days)
or stored below -18 ° C (no more than 12months)
or stored below -70° C (long-time storage)
Samples can be transported between 2 to 35 °C in 2 hours.
The sample should be transported by curing with ice or sealing with foam boxes and ice packs. The frozen samples should be thawed at room temperature. Please avoid repeated freezing and thawing.

6. Instructions for Use

6.1 Preparation of the Reaction Mix（Reagent preparation zone）

6.1.1 Take PCR Reaction Mix1 and 2 out from the kit, thawed at room temperature, vortex lightly and briefly centrifuge for use.

6.1.2 Prepare PCR Reaction buffer based on number of Samples (n) and add 40μL PCR Mix into each reaction tubes (sum to n+2 tubes), of which '2' refers to quality control 1 and quality control 2.

6.2 Sample Preparation（Sample preparation zone）

Preparation: Take quality control 1 and quality control 2 out from the kit, thawed at room temperature vortex lightly and briefly centrifuge for use.

It is recommended to use a validated nucleic acid extraction reagent (Record No.: Sutong Machinery No. 20180035 or Sutong Equipment No. 20190002), and perform nucleic acid extraction according to the instructions.

6.2.1 Nucleic acid extraction reagent (Record No.: Sutong Machinery No. 20180035) was used to extract nucleic acid according to the instructions.

(1) Transfer 200μL of oral exfoliated cell or 200μL of the whole blood to a 1.5 mL tube containing 1000μL of normal saline, centrifuge at 12000 rpm for 2 minutes, and discard the supernatant using a pipette.

(2) Add 1 mL of normal saline to the 1.5 mL tube, shake until there is no visible precipitate, centrifuge at 12000 rpm for 2 minutes, and discard the supernatant using a pipette.

(3) Add 100μL quality control 1and quality control 2 to two new 1.5ml tubes separately.

(4) Add 100μL DNA extraction buffer to tubes with sample (6.2.1-2), and tubes with quality control 1 and 2 (6.2.1-3).

Notes: DNA extraction solution should be mixed upside down timely before and during use.

(5) Scatter the sediment by the vortex shaker. Put it into 90°C dry bath or water bath for 10±2 minutes. Centrifuge at 12000rmp for 2 minutes, and the supernatant is ready for PCR assay.

6.2.2 Nucleic acid extraction reagent (Record No.: Sutong Machinery No. 20190002) was used to extract nucleic acid according to the instructions.

(1) Transfer 200μL oral exfoliated cell and 200μL whole blood to 1.5ml tubes separately. Transfer 100μL quality control 1 and 100μL quality control 2 to 1.5ml tubes separately. Add 100μL normal saline into each quality control.

(2) Add 200μL Lysis Solution and 20μL of proteinase K to the samples, vortex thoroughly and heat at 70°C for 10 minutes.

(3) Add 230μL of absolute ethanol to the 1.5mL tube, mix the solution, transfer it to a nucleic acid adsorption column and centrifuge at 10000g for 1 minutes

(4) Add 700μL wash buffer 1 into a new column and centrifuge at 10000g for 1 minute.

(5) Discard the waste, add 500μL wash buffer 2 and centrifuge at 10000g for 1 minute.

(6) Change a new column, centrifuge at 12000g for 2 minutes and discard the column.

(7) Insert the nucleic acid adsorption column in to a 1.5mL tube, add 200μL eluent, and incubate at 70°C for 2 minutes.

(8) centrifuge at 12000g for 2 minutes and the solution is ready for the assay.

Notes: The DNA sample is deserved to PCR immediately, or stored below -18 °C (no more than 18 months, no more than 3 times of freezing and thawing). When used again, the extracted DNA should be fully melted, mixed and centrifuged transiently. The supernatant can be taken for PCR assay.

6.3 Sample Adding（Sample preparation zone）

Add quality control 1, quality control 2 and the tested sample (5μL each) into the prepared PCR tube. After capping the tube tightly, centrifuge in a low speed transiently.

Notes: Do not touch the sediment at the bottom of the tube when the sample is pipetted.

6.4 PCR Amplification（PCR amplification zone）

6.4.1 Sample serial number: According to the sample types, sample, quality control 1, and quality control 2 number were set.

6.4.2 Fluorescence Channel Selection:

The SY255, SRY, SY127, SY84, and ZFX amplification were detected at FAM, JOE, TEXAS RED, CY5, and TAMRA channel respectively in PCR reaction solution 1. The quenched fluorescence and the passive reference fluorescence were set to None.

The SY254, SRY, SY134, SY86, and ZFX amplification were detected at FAM, JOE, TEXAS RED, CY5, and TAMRA channel respectively in PCR reaction solution 2. The quenched fluorescence and the passive reference fluorescence were set to None.

6.4.3 Reaction conditions setting:

	steps	Temperature (°C)	Time (seconds)	cycles
1	UDG enzyme reaction	37	300	1
2	UDG enzyme inactivation, pre-denaturation	95	300	1
3	denaturation	95	10	40
	Anneal, extend and detect fluorescence	60	30	
The detection channels of PCR reaction solution 1 and 2 were all of FAM, JOE, TEXAS RED, CY5 and TAMRA.				

Reaction volume: 50μL

6.4.4 Save files, run program

6.5 Data Analysis

After the reaction completed, set the baseline within 3-15 (Baseline cycle can vary within a certain range according to the actual situation). The threshold setting principle is that the threshold line just exceeds the highest point of the negative control amplification curve (irregular noise line), and the threshold cycle (Ct) value is displayed as 'Undetermined'. The general Y chromosome threshold is 1.0E+04-2.0E+05. It is recommended to set the threshold of Y chromosome SY84 and SY86 to 4.0E+04, the threshold of SY127, SY134, SY254 and SY255 to 5.0E+04, and the threshold of SRY and ZFX to 2.0E+04. The results were automatically analyzed by the instrument software.

6.6 Quality Control

6.6.1 Quality control 1: The SY84, SY86, SY127, SY134, SY254, SY255, SRY, and ZFX PCR reaction had typical S-type amplification curves, and the Ct value is ≤ 36.00.

6.6.2 Quality control 2: The SY84, SY86, SY127, SY134, SY254, SY255, and SRY PCR reaction had no typical S-type amplification curve, and the Ct value is shown as 'Undetermined'. The ZFX PCR reaction had typical S-type amplification curve, and the Ct value is ≤ 36.00.

6.6.3 The result for positive and negative control must conform to explanation in 6.6.1 and 6.6.2, otherwise PCR failure and you need repeat this experiment.

7. Reference Range

The Ct value of any one of SY84, SY86, SY127, SY134, SY254, SY255, and SRY PCR reaction was shown as 'Undetermined', and the Ct value of ZFX PCR reaction were all ≤ 36.0, which means that the Y chromosome detection site was negative.

8. Interpretation of Data

On the premise that the experiment was effective, the judgment of Y chromosome deletion negative or positive are follows.

8.1 The Ct value of SY84, SY86, SY127, SY134, SY254, SY255, SRY, and ZFX PCR reaction, and the Ct value of ZFX PCR reaction was ≤ 36.0, which means that the Y chromosome detection site was negative.

8.2 Any Ct value of SY84, SY86, SY127, SY134, SY254, SY255, and SRY detections is shown as 'Undetermined' or Ct value is more than 36, at the same time, ZFX Ct value is no more than 36.0 means that the Y chromosome detection site was positive. A single deletion of SY254 or SY255 was usually caused by experimental errors. A retest was recommended to determine if it was a positive deletion of the Y chromosome.

8.3 Any Ct value of SY84, SY86, SY127, SY134, SY254, SY255, and SRY PCR reaction is shown as 'Undetermined' or Ct value is more than 36, at the same time, ZFX Ct value is more than 36, which means the sample concentration was not enough and a resampling test was recommended.

9. Procedural Limitations

9.1 This kit was suitable for clinical auxiliary diagnosis. The clinical diagnosis and treatment of patients should be considered in combination with other medical examinations.

9.2 False negative or false positive results may occur when samples were collected, handled, transported, and stored improperly.

9.3 Possibility analysis of false negative results: samples were collected, handled, transported, and stored improperly; the sample concentration was not enough; sequence variation of the target sequence to be measured or sequence variation caused by other factors.
The possibility of false negative results can be reduced by sampling the same patient at different stages and multiple sites.

5.4 The kit can be only used for the diagnosis of Y-chromosomal microdeletions of the AZFa (sY84 and sY86), AZFb (sY127 and sY134), and AZFc (sY254 and sY255) region.

5.5 The concentration of acceptable interfering substances was 0.05 mg/mL hemoglobin and 100 μg/mL mucin.

10. Performance Indexes

10.1 The minimum detection limit of this kit can reach 1 ng/μL of human genomic DNA.

10.2 The coincidence rate was 100% when tested by negative and positive corporate reference products.

10.3 The variation coefficient of intra-assay precision of this kit is less than 5%.

11. ⚠ Warnings and Precautions

11.1 Read this instruction carefully before using. The operator should have received professional training in gene amplification or molecular biology testing, and have relevant qualifications. The laboratory should have reasonable biosafety preparedness facilities and protective procedures.

11.2 The entire experimental process should be carried out in three areas (reagent preparation area, sample processing area, PCR amplification area). These three areas are independent. Equipment, supplies and work clothes are dedicated for use in designated zones without cross-use; Please clean the workstations immediately after the experiment.

11.3 The product should be fully melted at room temperature, mixed and centrifuged at low speed transiently.

11.4 The quality controls should be set at each test. Do not mix reagents of different batches. Use the kit before the expiry day.

11.5 The samples should be as fresh as possible, and the extraction process should be strictly protected against DNA enzyme contamination and DNA degradation caused by improper operation.

11.6 Try to avoid air bubbles when dispensing the reaction solution. Before placing it into the instrument, check whether the PCR tubes are tightly closed to avoid contamination of the instrument and the environment.

11.7 The tested samples should be seen as contagious substances. The operation and handling of it should meet the requirements of related regulations.

12. References

12.1 EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013.