RepoDx®

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MTHFR (C677T) DNA qPCR Detection Kit
Package insert
For In Vitro Diagnostic Use Only

REF

32

-18℃

IVD

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

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1. Intended Use

MTHFR is the key enzyme in the folate metabolism, its gene polymorphism can lead to the decrease of the key enzyme activities in folate metabolism and cause the disorder of folic acid metabolism, which leads to many diseases. In addition, the MTHFR polymorphism affects the therapeutic effects of 5-FU and methotrexate (MTX).

The kit can be used for the diagnosis of MTHFR (methylenetetrahydrofolate reductase) gene locus C677T polymorphism in human whole blood or human oral exfoliated cells. 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: Detection of MTHFR (C677T) locus polymorphism.

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 dual-labeled oligonucleotide detection probe specific to the target DNA. The Master Mix reagent contains primers specific to MTHFR C677T gene site. The detection of amplified DNA is performed using target specific dual-labeled oligonucleotide probes that permit independent identification of CC, CT and TT amplicon.

3. Kit Contents

Label	Main Compositions	Number	Volume /tube
MTHFR PCR Mix	Taq, UDG, Probes, PCR buffer, dNTPs, Mg^{2+}	1	1.44ml
MTHFR Positive control 1	40000 copies/μL MHTFR (677CC) wild type plasmid	1	0.20ml
MTHFR Positive control 2	40000 copies/μL MHTFR (677TT) mutant type plasmid	1	0.20ml
MTHFR Positive control 3	20000 copies/μL MHTFR (677CT) Heterozygous type plasmid	1	0.20ml
MTHFR Negative control	ddH_2O	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 Sample source: human EDTA anticoagulant whole blood, human sodium citrate anticoagulant whole blood, or human oral exfoliated cells.

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 24 hours)
or stored below -18 ° C (no more than 12months)
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 MTHFR PCR Mix 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 45μL MTHFR PCR Mix into each reaction tubes (sum to n+4 tubes), of which '4' refers to MTHFR positive control 1, positive control 2, positive control 3 and negative control.

6.2 Sample Preparation（Sample preparation zone）

Preparation: Take MTHFR positive control 1, positive control 2, positive control 3 and negative control 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 50μL 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 50μL MTHFR positive control 1, positive control 2, positive control 3 and negative control to four new 1.5ml tubes separately.

(4) Add 50μL DNA extraction buffer to tubes with sample (6.2.1-2), and tubes with positive and negative controls (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, 50μL whole blood, MTHFR positive control 1, positive control 2, positive control 3 and negative control to 1.5ml tubes separately.

Add 200μL Lysis Solution and 20μL of proteinase K to the sam - ples, vortex thoroughly and heat at 70°C for 10 minutes.

(2) 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.

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

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

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

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

(7) 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 MTHFR negative control, MTHFR positive control 1, 2, 3 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 Experiment type: Genotyping

6.4.2 Fluorescence setting: SNP Assay Name: C677T
Allele 1Name or Base (s): C, Reporter: VIC, Quencher: NFQ-MGB;
Allele 2Name or Base (s): T, Reporter: FAM, Quencher: NFQ-MGB.
Passive Reference: None.

6.4.3 Sample setting: Sample serial number: According to the sample types, sample number were set. Set the task of MTHFR positive control 1 as Positive Control Allele 1/ Allele 1, set the task of MTHFR positive control 2 as Positive Control Allele 2/ Allele 2, set the task of MTHFR positive control 3 as Positive Control Allele 1/ Allele 2.
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	
MTHFR C677: FAM MTHFR 677T: VIC				

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 MTHFR threshold is 2.0E+04-2.0E+05, and the general MTHFR threshold is 1.0E+04-1.0E+05. It is recommended to set the threshold of MTHFR to 5.0E+04. The results were automatically analyzed by the instrument software.

6.6 Quality Control

6.6.1 Negative control: The C and T PCR reaction had on typical S-type amplification curves, and the Ct value is shown as 'Undetermined'.

6.6.2 Positive control 1: The C PCR reaction had typical S-type amplification curve, and the Ct value is ≤ 34.00; the apply call is shown as C/C.

6.6.3 Positive control 2: The T PCR reaction had typical S-type amplification curve, and the Ct value is ≤ 34.00; the apply call is shown as T/T.

6.6.4. Positive control 3: The C and T PCR reaction had typical S-type amplification curve, and the Ct value is ≤ 34.00; the apply call is shown as C/T.

6.6.5 The result for positive and negative control must conform to explanation in 6.6.1, 6.6.2, 6.6.3 and 6.6.4, otherwise PCR failure and you need repeat this experiment.

7. Reference Range

7.1 If the apply call is shown as C/C, the result is considered as MTHFR 677 gene locus homozygous wild type.

7.2 If the apply call is shown as T/T, the result is considered as MTHFR 677 gene locus homozygous mutant type.

7.3 If the apply call is shown as C/T, the result is considered as MTHFR 677 gene locus heterozygous mutant type.

8. Interpretation of Data

On the premise that the experiment is effective, the judgment of MTHFR genotype are as follows.

8.1 If the apply call is shown as C/C, the result is considered as MTHFR 677 gene locus homozygous wild type (C/C).

8.2 If the apply call is shown as T/T, the result is considered as MTHFR 677 gene locus homozygous mutant type (T/T).

8.3 If the apply call is shown as C/T, the result is considered as MTHFR 677 gene locus heterozygous mutant type (C/T).

8.4 If the apply call is shown as 'Undetermined', the result determination should be compared between MTHFR 677 gene locus and positive controls (1, 2, 3). If the result cannot be determined, the assay should be re-carried out.

9. Procedural Limitations

9.1 The kit is used for clinical auxiliary diagnosis. The clinical treatment of the patients should be combined with other medical examinations.

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

9.3 The concentration of acceptable interfering substances: 0.05 mg/mL hemoglobin, 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%.

10.4 The specificity test showed that the accuracy of MTHFR C677T was 100% by using sequencing method.

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 positive and negative 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 Peadar N Kirke, James L Mills, Anne M Molloy,et al. *Impact of the MTHFR C677T polymorphism on risk of neural tube defects: case-control study*. BMJ. 2004;328:1535-1536.

12.2 S. Afzal, S. A. Jensen, B. Vainer, et al. *MTHFR polymorphisms and 5-FU-based adjuvant chemotherapy in colorectal cancer*. Annals of Oncology. 2009;20: 1660–1666.