MTHFR Qualitative Real-Time PCR Kit

cedbio

For Professional Use Only RUO -20°C

S No.	REF	Reactions
1	NMFR24004_02	20
2	NMFR24004_01	50
3	NMFR24004	100

Expiry Date - See box label for information

Please read this IFU carefully before starting.

INTENDED USE

MTHFR Qualitative Real-Time PCR kit is a qualitative assay for the detection and differentiation of MTHFR mutations (C677T and 1298C) in human genomic DNA extracted from EDTA whole blood. LNA probes used in this assay specifically detects and differentiate wild and mutant alleles.

BACKGROUND

Methylenetetrahydrofolate reductase (MTHFR) plays a central role in folate and homocysteine metabolism by catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate which is utilized in homocysteine remethylation to methionine. Several Single Nucleotide Polymorphisms (SNPs) have been identified that affect folate and homocysteine metabolism, which in turn are implicated in the pathogenesis of Cardiovascular disease, neural tube defects, and colorectal cancers. These SNPs include MTHFR C677T, MTHFR A1298C, Methionine Synthase (MTR) A2756G and Methionine Synthase Reductase (MTRR) A66G. The common gene variants are MTHFR C677T and A1298C. In the MTHFR gene, the C677T polymorphism occurs in exon region, which involves a C to T substitution at position 677, a consequence of transformation from an alanine to a valine at codon 222 in the N-terminal catalytic domain.

Individuals with the 677TT homozygous variant have no more than 30 % of normal enzyme activity, and heterozygotes CT genotype have 65% of normal enzyme activity and increased thermolability. An additional polymorphism, MTHFR A1298C, occurs in exon region resulting into the change from a glutamic acid to alanine residue at codon 429 in the C-terminal regulatory domain of the protein and decreases the enzyme activity.

COMPATIBLE INSTRUMENTS

Real-Time PCR, instruments like, BIORAD - CFX96, THERMO -QS5, QIAGEN-ROTOR - GENE Q and other instruments which supports FAM (495 nm - 520 nm), HEX (535 nm - 556 nm), Cy5 (649 nm - 670 nm) and ROX (575 nm - 602 nm).

PRECAUTIONS

- Treat all the specimens as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Keep separate area for master mix and template preparation and work under biosafety cabinets.
- Use aerosol barrier pipette tips and frequently change the gloves.

STORAGE AND HANDLING

- Store all MTHFR Qualitative Real-Time PCR Detection Kit reagents at -20°C.
- Do not repeatedly freeze-thaw reagents more than 5 times it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C. Recommended freeze thaw cycle time is 5 times.
- Kit components are stable through the end of the expiration date indicated on the box when stored at -20°C. Shelf Life - 12 Months from date of manufacturing.

MATERIALS REQUIRED BUT NOT PROVIDED

Consumables

PCR Plates/tubes

PCR plate covers/tube caps

KIT CONTENTS

Kit Components	100 Rxns Vol.	50 Rxns Vol.	20 Rxns Vol.
Master Mix	4 x 625 µL	2 x 625 µL	500 µL
Primer and Probe mix A	125 µL	62.5 µL	25 µL
Primer and Probe mix B	125 µL	62.5 µL	25 µL
Nuclease Free Water	1500 µL	750 µL	300 µL
Positive Test Control	200 µL	100 µL	40 µL
Instruction for use	1 Nos	1 Nos	1 Nos

Table 1:- Kit Content

FLUORESCENT PROBE DETAILS

Tube	Target	Reporter	Quencher
Tube A	C677T Wild	HEX/VIC	BHQ2
	A1298C Mutant	Texas red	BHQ2
Tube B	C677T Mutant	FAM	BHQ1
	A1298C Wild	Cy5	BHQ2

Table 2:- Target,Reporter and Quencher

*Note: In some instruments where the BHQ option is unavailable, please set the quencher to "NONE."

REACTION MIXTURE 25 µL

Reagents	1 Rxn	20 Rxns	50 Rxns	100 Rxns
Master Mix	12.5 µL	250 µL	625 µL	1250 µL
Primer & Probe Mix Tube A or Tube B	1.25 µL	25 µL	62.5 µL	125 µL
Nuclease Free Water	6.25 µL	125 µL	312.5 µL	625 µL
Total	20 µL	400 µL	1000 µL	2000 µL

Table 3:- Reaction Mixture

Use 5 µL of the test DNA (10 ng/µL) and Positive Test Control (PTC)

REACTION SET-UP

DNA should be extracted from human EDTA whole blood. It is recommended to use a commercially available Human Blood DNA extraction kit for the extraction. For example, Cedbio' s Blood DNA extraction kit or Qiagen's QIAamp DNA Kit. Store the extracted DNA at -20 ℃ for further use.

REACTION SET-UP

- Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
- Calculate the number of reactions for each experiment including all controls with one excess reaction volume in the reaction cocktail to accommodate pipetting errors (eg: number of reaction (n) including controls are 10 add 1 extra reaction during the preparation n+1).
- Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples referring the table no. 3, in Master Mix Preparation room.
- Assay should be run along with positive controls and negative controls.
- Refer to table no. 3, to prepare the reaction mixture of 25 µL.
- Calculate the number of the samples to be tested along with the controls and multiply with 1 reaction volume extra to get the final reaction mixture.
- Spin down the tubes and dispense 20 µL reaction mix in each tube strips or 96 well plate.
- Before moving to template adding area, add 5 µL of nuclease free water in NTC well.
- Carefully add 5 µL DNA samples kept on ice in the designated wells in template addition room. Add 5 µL of PTC.The assay should be run along with positive controls and negative controls.

- Seal the plate carefully, briefly spin down and use any qRT-PCR instrument which complies with the dyes specified in the kit insert.

THERMAL CYCLING CONDITION

STEPS	TEMP (° C)	TIME	QUANTITATION	CYCLE
Hold	95	1 min	Off	1
PCR and Quantitation	95	5 sec	Off	40
	63	20 sec	On	

Table 4:- Thermal Cycling Condition

READING TEST RESULTS / DATA ANALYSIS

- NTCs should be negative and should not exhibit fluorescence amplified curves that cross the threshold line.
- If a false positive occurs with one or more of the primer and probe in NTC reactions , it indicates sample contamination.
- In that case, Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.
- Positive control should produce a positive result with an expected Ct value for each target included in the test.
- If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines.
- After completion of the run, analyze the data as per the instrument manufacturer instructions.
- Analysis should be performed separately for each target using a manual threshold settings.
- In case internal control has not worked for a sample re-do the test with 2 or more dilutions.
- Negative results do not exclude possibility of infection and should not be used as the sole basis for the treatment.

RESULT INTERPRETATION

For Target Gene	Assay Result
Ct < 37	Positive
Ct = Undetermined or Ct ≥ 37	Negative
Ct = Undetermined or Ct ≥ 37	Invalid. Re-purify the nucleic acid from the sample, then repeat the test.

DATA INTERPRETATION

C677T Wild (HEX/VIC)	C677T Mutant (FAM)	Results for C677T
+	+	Mutant Heterozygous C/T
+	-	Wild Homozygous C/C
-	+	Mutant Homozygous T/T
-	-	Invalid, repeat the DNA extraction

A1298C Wild (CY5)	A1298C Mutant (Texas Red/ROX)	Results for A1298C
+	+	Mutant Heterozygous A/C
+	-	Wild Homozygous A/A
-	+	Mutant Homozygous C/C
-	-	Invalid, repeat the DNA extraction

Target	Ct cutoff for PTC
C677T WILD	20-21
C677T MUTANT	17-18
A1298C WILD	23-24
A1298C MUTANT	21-22

WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation & expired kit components along with bio-waste as per the lab manual/general bio-waste management instruction.
- Dispose the PCR plates with patient samples “sealed” post run to avoid potential infection to the operators and contamination of the lab.

TROUBLESHOOTING

Positive control showed no amplification

Inappropriate storage of reagents

- *Store the reagents at recommended temperature for their optimal performance.*
- *Avoid repetitive freezing and thawing.*
- *Check the expiry of reagents.*

Negative controls are positive

Causes - Cross-Contamination

- *Follow good laboratory practices to avoid contamination issues.*
- *Use a new batch of reagents and repeat the experiment.*

Abnormal plot and/or low ΔRn values in amplification curve

The baseline was set improperly (some samples have CT values lower than the baseline value)

- *Switch from manual to automatic baseline, or move the baseline stop value to a lower CT (2 cycles before the amplification curve for the sample crosses the threshold).*

An amplification signal is detected in the early cycles

Dilute the sample to increase the CT value.

SYMBOL	MEANING
RUO	Research use only
REF	Product Code
LOT	Lot Number
-20°C	Store at -20ºC
Σ	Contains sufficient for <n> reaction
	Manufacturer
	Date of Manufacture (MMM, YYYY)
	Expiry Date (MMM, YYYY)
	Instruction for use

MANUFACTURED By

Cedbio Biotech Labs Pvt Ltd

Hall9, Iran Packing Industries Company,
Kilometer 8 On Fath Expressway, Tehran, Iran

WWW.Darmannegar.com Info@darmannegar.com