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Human MNBH Gene Real Time PCR Kit
User Manual
For In Vitro Diagnostic Use Only

REF **QD-0238-02**

Σ 25 −20 °C IVD

For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4; LightCycler®480 Instrument

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1. Intended Use

Human MNBH real time PCR kit is used for the detection of MNBH gene by using real time PCR systems in human samples like blood, tissue, swab, cells et al.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Minibrain (MNBH) is also known as DYRK1 and is the human homolog of the mnb gene in Drosophila and is a single copy gene mapping on human chromosome 21q22.2. MNBH is considered a proper internal control gene for human samples used in PCR detection.

Human MNBH gene real time PCR kit contains a specific ready-to-use system for the detection of the MNBH gene by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the MNBH DNA. Fluorescence is emitted and measured by the real time systems´ optical unit. The detection of amplified MNBH DNA fragment is performed in fluorimeter **channel FAM**. DNA extraction buffer is available in the kit.

4. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	MNBH Reaction Mix	1 vial, 950µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	MNBH Positive Control	1 vial, 60µl

Analysis sensitivity: 1×10^3copies/ml; LOQ: 2×10^3～1×10^8copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided.
- Cool all reagents during the working steps.
- Super Mix and Reaction Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and Freezer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

7. ⚠Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols.

8. Sample Collection, Storage and transport

- Collected samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

9.1.1 Tissue samples

1) Wash the tissue with sterile saline for several times.
2) Take 50mg sample in a tube, add 1ml sterile saline, and grind the tissue into homogenate.
3) Transfer the homogenate to a 1.5ml tube, and centrifuge the tube at 13000rpm for 5min. Remove the supernatant, and keep the sediment for processing.
4) Add 100µl DNA extraction buffer in the tube (sediment), close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
5) Incubate the tube for 10 minutes at 100°C.
6) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.2 Liquid samples

1) Pipet 100µl sample to a 0.5ml tube, add 100µl DNA extraction buffer, close the tube and vortex for 10 seconds. Spin down briefly in a table centrifuge.
2) Incubate the tube for 10 minutes at 100°C.
3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and is used for PCR template.

9.1.3 Swab samples

1) Wash the genital swabs in 1.0ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 1.0ml normal saline and suspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
3) Add 50µl DNA extraction buffer, close the tube then suspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.
4) Incubate the tube for 10 minutes at 100°C.
5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

Attention:

A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination in case the sample is positive.
B. The extraction sample should be used in 3 hours or stored at -20°C for one month.
C. DNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For DNA extraction, please comply with the manufacturer's instructions.

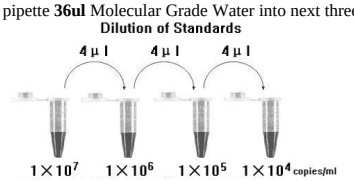
9.3 Quantitation

The kit can be used for **quantitative** or **qualitative** real-time PCR.

For performance of quantitative real-time PCR, standard dilutions must be prepared firstly as follows. Molecular Grade Water is used as the dilution.

Dilution is not needed for performance of qualitative real-time PCR detection.

Take positive control (1×10^7copies/ml) as the starting high standard in the first tube. Respectively pipette **36ul** Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



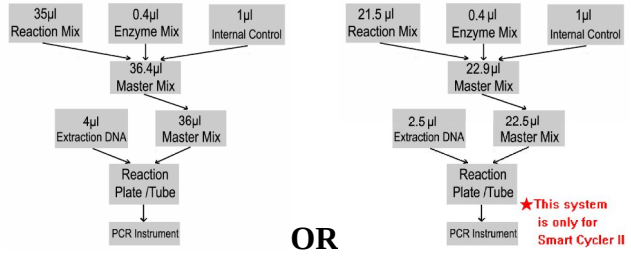
To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

Attention:

A. Mix thoroughly before next transfer.
B. The positive control (1×10^7copies/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

9.4 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



※PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **36µl (22.5µl for SmartCycler II)** Master Mix with micropipets of sterile filter tips to each real time PCR reaction plate/tubes. Separately add **4µl (2.5µl for SmartCycler II)** DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

37°C for 2min	1cycle
94°C for 2min	1cycle
93°C for 15sec, 60°C for 1min (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
FAM	Target Nucleic Acid

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher**.

10. Threshold setting: just above the maximum level of molecular grade water.

11. Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12. Quality control: Negative control, positive control, and QS curve must be performed correctly, otherwise the sample results is invalid.

Channel / Control	Ct value of Channel FAM
Molecular Grade Water	UNDET
Positive Control(qualitative assay)	≤35
QS（quantitative detection）	Correlation coefficient of QS curve≤－0.98

13. Data Analysis and Interpretation: The following sample results are possible:

	Ct value In Channel FAM	Result Analysis
1#	UNDET	It can be considered a failure of DNA extraction from the sample
2#	≤38	The sample contains MNBH, and it can be considered a success of DNA extraction from the sample
3#	38～40	Re-test; if it is still 38~40, report as 1#

For further questions or problems，please contact our technical support at **trade@liferiver.com.cn**