1. Intended Use

By using real time PCR systems, Coxsackie Virus real time PCR kit is used for the detection of Coxsackie Virus in samples like nasal and pharyngeal secretions, sputum, provoked sputum, stool, C.S.F. and etc.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5′nucleic 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 fluorescent signal is detected is initially 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

Coxsackie Virus is a cytoplasmic virus of the Picornaviridae family, an enterovirus (a group containing the polioviruses, coxsackieviruses, and echoviruses). There are 61 non-polio enteroviruses that can cause disease in humans, of which 23 are Coxsackie A viruses (6 are Coxsackie B viruses). Enteroviruses are the second most common viral infectious agents in humans (after the rhinoviruses). The most well known Coxsackie A disease is hand, foot and mouth disease (unrelated to foot and mouth disease), a common childhood illness, often produced by Coxsackie A16. Other diseases include acute haemorrhagic conjunctivitis (A24 specifically), herpangina, and aseptic meningitis (both Coxsackie A and B viruses). The genome is single-stranded positive-sense RNA genome that is about 7500 nucleotide long. The viral particle is about 30nm in diameter with icosahedral symmetry.

4. Kit Contents

- Coxsackie Virus real time RT-PCR kit contains a specific ready-to-use system for the detection of the Coxsackie Virus (for 23 species of type A and 6 species of type B) using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains a Super Mix for the specific amplification of the Coxsackie Virus RNA. The reaction is done in one step real time PCR. The first step is a reverse transcription (RT), during which the Coxsackie Virus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems’ optical unit during the PCR reaction. The PCR kit contains a specific ready-to-use system for the detection of the accumulating product without having to re-open the reaction tube after the amplification.

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, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices

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

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, which 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.

8. Sample Collection, Storage and Transport

- Collected samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

9. Procedure

9.1 RNA-Extraction

Different brands of RNA extraction kits are available. You may use your own extraction systems or the commercial kit based on the yield. For RNA extraction kit, please comply with manufacturer’s instructions. The recommended extraction kit is as follows:

9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal control (IC) allows the user to determine and control the possibility of PCR inhibition.

9.3 RT-PCR Protocol

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

1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls 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 15µl Master Mix with micropipets in sterile filter tips to each real time PCR reaction plate/tubes. Separately add 5µl RNA 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:

10. Threshold setting:

Choose Arithmetic as back ground and none as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

11. Quality control:

Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

12. Data Analysis and Interpretation

The following sample results are possible:

Crossing point value

Result Analysis

Below the detection limit or negative

Positive

Re-test; if it is still 38–40, report as 1º

Blank

PCR Inhibition, No diagnosis can be concluded.