CERTIFICATE OF ANALYSIS

Cy5.5, SE

Catalog #: 1057

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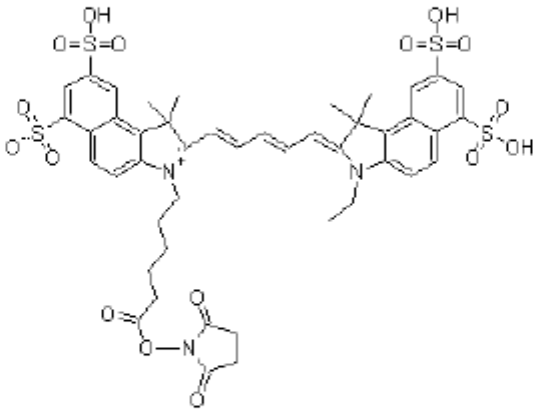
Product Name	Cy5.5, SE
Molecular Formula	$C_{45}H_{44}K_3N_3O_{16}S_4$
Molecular Weight	1128.42
Solubility	Soluble in DMSO and DMF
Chemical Structure	

Items	Lot #LJZ004420	Specifications (≥1 mg) #1057
Appearance	Blue powder	Blue powder
Absorption Maximum	689.8nm	690±5 nm
Extinction Coefficient	N/A	N/A
HPLC Purity	95.66%	≥ 95% @254 nm detection
Storage Condition	-20 °C, and desiccated	-20 °C, and desiccated
Shipping Condition	Room temperature	Room temperature

Gentaur Molecular Products
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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

For In Vitro Diagnostic Use Only

1. Intended Use
Crimean-Congo hemorrhagic fever virus (CCHFV) Real Time RT-PCR Kit is used for the detection of

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Crimean-Congo hemorrhagic fever virus (CCHFV) Real Time RT-PCR Kit is used for the detection of Crimean-Congo hemorrhagic fever virus in serum, plasma or insect vector by using real time PCR systems.

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

Crimean-Congo hemorrhagic fever virus (CCHFV) (genus Nairovirus, family Bunyaviridae) causes severe disease with a fatality rate as high as 30%. The virus is transmitted to humans by the bite of ixodid ticks (primarily of the Hyalomma genus) or by contact with blood or tissues from infected persons or infected livestock. The risk for spread of the virus from person to person is high, which occasionally results in nosocomial outbreaks. After an incubation period of 3 to 7 days, the patient has sudden onset of fever, chills, myalgia, and headache, which rapidly progress to severe illness; a hemorrhagic state follows with bleeding from the mucous

membranes and petechiae, associated withthrombocytopenia and leukopenia.

The CCHFV real time RT-PCR Kit contains a specific ready-to-use system for the detection of the CCHFV 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 CCHFV RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the CCHFV 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. The detection of amplified CCHFV DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). An external positive control defined as 1×107copies/ml is supplied which allow the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

4. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	CCHFV Super Mix	1 vial, 480µl
2	RT-PCR Enzyme Mix	1 vial, 28µl
3	Molecular Grade Water	1 vial, 400µl
4	Internal Control (IC)	1 vial, 30µl
5	CCHFV Positive Control(1×10^7copies/ml)	1 vial, 30µl

Analysis sensitivity: 1×103copies/ml LOQ: 2×103～1×108copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the RNA extraction kits recommended, 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, 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
• RNA 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 raks

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.
• Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

9. Procedure

9.1 RNA-Extraction

RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction,

please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
RNA Isolation Kit	ME-0010/ME-0012	ZJ Biotech
QIAamp Viral RNA Mini extraction Kit (50)	52904	QIAGEN