



EliGene® HSV1/HSV2 UNI

REF 90062-UNI (for 50 samples)

Kit components:

5 x 150 µl HSV1 UNI Mix
5 x 150 µl HSV2 UNI Mix
2 x 500 µl IC DNA 01
2 x 20 µl Eli Blocker
2 x 20 µl ROX dye
1 x 100 µl HSV1/HSV2 QS1 (1 x 10² cop/µl)
1 x 100 µl HSV1/HSV2 QS2 (1 x 10³ cop/µl)
1 x 100 µl HSV1/HSV2 QS3 (1 x 10⁴ cop/µl)
1 x 100 µl HSV1/HSV2 QS4 (1 x 10⁵ cop/µl)
1x Instruction for use

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

Kit EliGene® HSV1/HSV2 UNI is intended for detection of pathogenic species of herpesvirus 1 (Herpes simplex virus 1 – HSV1) and herpesvirus 2 (Herpes simplex virus 2 – HSV2) in clinical samples.

Principle of the method

This diagnostic kit is based on RealTime PCR method. In this kit primers and fluorescent probes (FAM and HEX) for the detection of HSV1/HSV2 DNA and internal control are used.

Introduction

Herpesvirus is enveloped DNA virus. To date, at least eight different herpesvirus species are known to infect man. HSV1 is enveloped DNA virus. Today, at least eight different herpesvirus species are known to infect man. A primary infection typically occurs during the childhood. The primary infection goes often through symptomless. The HSV1 infection can manifest as an undifferentiated catarrh of respiratory system of adults or older children. The infection of the conjunctiva and cornea manifests as keratoconjunctivitis. HSV1 is a neurotropic virus. The DNA diagnostics of HSV1 is applicable for samples (swabs) from cornea, conjunctiva, skin lesions and blood or from the cerebrospinal fluid.

HSV2 causes genital herpes, characterized by sores in the area of the genitalia. Genital herpes is a sexually transmitted disease. The main symptoms are herpes ulcers in glans penis in men. Lesions in woman are localized in cervix, vagina or perianal area. The activation of the latent infection can produce asymptomatic releasing of virus in cervical secret. HSV2 can cause infection of the brain (encephalitis) in the case of severely defective or compromised immune system. Clinical samples for the DNA diagnostics of HSV2 include samples (swabs) from skin/mucosa lesions, blood or from the cerebrospinal fluid (CSF).

Primary sample collection, handling and storage

Clinical material:

Serum, plasma, CSF, swabs

Recommended DNA isolation procedure:

Manual: EliGene® Urine Isolation Kit (ELISABETH PHARMACON)
Automatic: Prepito NA Body Fluid Kit (PerkinElmer)



Tissue, Biopsies

Manual: UltraClean Tissue DNA Kit (Qiagen)
Chemagic DNA Tissue10 Kit (PerkinElmer)

Automatic: Prepito DNA Tissue10 Kit (PerkinElmer)

Serum, Plasma, CSF:

According to standard protocol take the sample of the serum, plasma or CSF into sterile tubes. Samples must be stored and transported at 4 °C. For the diagnostic purposes, it is recommended to isolate DNA from the sample during the day of taking sample. For DNA isolation at least 200 µl of sample must be used.

Manual isolation:

Add 20 µl of Proteinase K to the sample and 10 µl of Internal Control DNA 01 (IC DNA 01) and then continue according to the standard protocol of EliGene® Urine Isolation Kit for DNA isolation from blood. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

Add 10 µl of Internal Control DNA 01 (IC DNA 01) and isolate DNA from the sample by using Prepito NA Body fluid kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Swabs:

These specimens should be collected by swabs according to standard protocol in collection tubes with transport medium (also 200-400 µl of physiological solution could be used) or MI3 buffer. Specimens should be transported to the laboratory at 4 °C in transport medium or use directly 400 µl of MI3 medium from EliGene Urine Isolation Kit for storage and transportation. **Do not use dry swabs!**

Manual isolation:

1. Use 200 µl of transport medium (or physiological solution with viruses) and add 200 µl of MI3 solution and add 20 µl of Proteinase K and 10 µl of Internal Control DNA 01 (IC DNA 01). Or, in the case that for transportation you used MI3 medium, add to 400 µl of MI3 solution - 20 µl of Proteinase K and 10 µl of Internal Control DNA (IC DNA 01).
2. Incubate tube 20 minutes at 56 °C in thermo shaker at 1000 rpm. Consequently shortly spin the tube.
3. By sterile pincers remove the swab and add 330 µl of solution MI4 to lysate. Vortex and shortly spin.
4. Continue according to the standard protocol of EliGene® Urine Isolation Kit. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

1. Into 2.0 ml tube pipette 450 µl of Lysis buffer, 200 µl of transport medium (or physiological solution with viruses), 10 µl of Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01).
2. Incubate tube 20 minutes at 56°C in thermo shaker at 1000 rpm. Consequently shortly spin the tube.
3. Remove the swab by sterile pincers, vortex and shortly spin.
4. Pipette all volume of sample to position H at Deep well plate from Prepito NA Body Fluid Kit.
5. Isolate DNA from the sample by using Prepito NA Body fluid kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Tissue, Biopsies:

Store the tissue immediately after the excise in the refrigerator at 4 °C and transport it to the laboratory during the same day.



Manual isolation:

For the diagnostic purposes, it is necessary to isolate DNA using UltraClean Tissue DNA Kit (MoBio) from the sample during the day of taking.

Add 10 µl of Internal Control DNA 01 (IC DNA 01) to the sample in the beating microtube. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

Cut up to 10 mg tissue sample into small pieces, add 200 µl Lysis Buffer, 6 µl Proteinase K and 10 µl of Internal Control DNA 01 (IC DNA 01) and isolate DNA from the sample by using Prepito DNA Tissue10 Kit according to protocol with elution to 50 µl of Elution buffer.

Additional required equipment

- Automatic pipette 5-20 µl and sterile tips with filter DNA RNA free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems LightCycler 2.0, LightCycler 480 a LightCycler Nano (Roche), Quant Studio 3 a 5 (ThermoFisher Scientific), RealTime System ABI 7500FAST (Applied Biosystems), Rotor-Gene 6000 nebo Rotor-Gene Q (Qiagen), CFX96 Touch Real-Time PCR Detection System (Bio-Rad), MIC qPCR Cyclor (Bio Molecular Systems).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

- For HSV1/HSV2 detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm)
- For Internal Control the probe labeled with HEX is used (similar to VIC – exc. 525 nm – em. 548 nm)

LightCycler® 2.0 (Roche):

For reaction use glass capillaries only.

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

95 °C	3 min	Ramp rate (20°C/s)	Acquisition mode "None"
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Step 2 - Analysis mode "Quantification", 50 Cycles

95 °C	5 s	Ramp rate (20°C/s)	Acquisition mode "None"
55 °C	20 s	Ramp rate (5°C/s)	Acquisition mode "Single"
72 °C	10 s	Ramp rate (20°C/s)	Acquisition mode "None"

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LC2_v00.ix0". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

LightCycler® 480 (Roche):

For reaction use white plates only. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.



In option Detection format choose “Dual Color Hydrolysis probe”.

Set up the following temperature profile:

Step 1 - Analysis mode “None”, 1 Cycle

95 °C 3 min Ramp rate (4.4°C/s) Acquisition mode “None”

Step 2 - Analysis mode “Quantification”, 50 Cycles

95 °C 5 s Ramp rate (4.4°C/s) Acquisition mode “None”

55 °C 20 s Ramp rate (2.2°C/s) Acquisition mode “Single”

72 °C 10 s Ramp rate (4.4°C/s) Acquisition mode “None”

Step 3 - Analysis mode “None”, 1 Cycle

40°C 1 min Ramp rate (2.2°C/s) Acquisition mode “None”

The complete temperature profile can be up-loaded from Run Template “EliGene_UNI_LC480_v00.ix0”. The Run Template can be imported to the software in menu “Navigator” by clicking to icon “Import” from the CD included in the kit.

QuantStudio 3 and 5 (ThermoFisher Scientific):

Use the Experiment type, “Presence/Absence”, Chemistry “TaqMan Probes”, and Run Mode “Standard”. As reporter dyes use FAM (HSV), VIC/HEX (IC DNA 01) and ROX as a passive reference dye.

Set up the following temperature profile:

Holding stage

95°C 3 min Ramp rate (1.6°C/s)

Holding stage

Cycling stage – 50 cycles

95°C 5 s Ramp rate (1.6°C/s)

55°C 20 s Ramp rate (1.6°C/s) Data collection ON

72°C 10 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 1 min Ramp rate (1.6°C/s)

Collect emission signal at the second step at 55 °C

The complete temperature profile can be up-loaded from Run Template “EliGene_UNI_QS3_v00.edt” or “EliGene_UNI_QS5_v00.edt”. The Run Template can be copied from the CD included in the kit.

LightCycler® Nano (Roche):

For reaction use clear strips only.

In “Run Settings” menu choose “Hydrolysis Probes” option and “High Quality” option.

In “Profile menu” set up the following temperature profile:

Step 1 - Hold

95 °C 3 min Ramp rate (5°C/s)

Step 2 – 3-Step Amplification, 50 cycles

95 °C 5 s Ramp rate (5°C/s)

55 °C 20 s Ramp rate (4°C/s) “Acquire” signal

72 °C 10 s Ramp rate (5°C/s)



Step 3 - Hold

40 °C 1 min Ramp rate (4°C/s)

In "Samples" menu click in window "Targets" (upper right window) on icon "+" and choose FAM dye as "Target 1". Then click once again on icon "+" and choose HEX dye as "Target 2". In window "Samples" (upper left window) click on icon "+" and add your samples. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LCNANO_v00.ppf". The Run Template can be copied from the CD included in the kit.

RealTime System ABI7500 (Applied Biosystems):

Use the program module for absolute quantification (Plate Type "Absolute Quantification" for ABI 7300, "Quantitation-Standard Curve" experiment for ABI 7500FAST). In case of usage ABI7500FAST instrument, use "7500 (96wells)" instrument type setting.

Set up the following temperature profile:

Holding stage

95 °C 3 min

Cycling stage – 50 cycles

95 °C 5 s

55 °C 20 s Data collection ON

72 °C 10 s

Collect emission signal at the second step – 55 °C.

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_ABI7500_v00.edt" or "HSV1HSV2_UNI_ABI7500_v00.edt". The Run Template can be copied from the CD included in the kit.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Startup Wizard Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:

Step 1 95°C 3 min

Step 2 95°C 5 s

Step 3 55°C 20 s + Plate Read

Step 4 72°C 10 s

Step 5 GOTO Step 2 50x

Step 6 40°C 20 s

Enter the Sample Volume 20 µl

Collect emission signal at the Step 3 at 55° C.

For filter settings use the "Scan Mode" All Channels but in Plate Manager select for the samples only fluorophores FAM and HEX. Then assign the samples with positions and Targets FAM and HEX as an "Unknown" sample or "Standard".

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_CFX96_v00.edt". The Run Template can be copied from the CD included in the kit.



Rotor-Gene 6000 or Q (Qiagen):

In the "New Run" window choose "Three Step" run
Choose the appropriate "Rotor Type" and click "Next".

Set up the following temperature profile:

Holding stage

95 °C 3 min

Cycling stage – 50 cycles

95 °C 5 s

55 °C 20 s Acquiring in channels "Green" and "Yellow"

72 °C 10 s

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_RG6000_v00.ret". The Run Template can be copied from the CD included in the kit.

MIC qPCR (Bio Molecular Systems Pty Ltd):

In Run Setup Create a new Run Profile for the MIC instrument. Enter the Sample Volume of 20 µl and Temperature Control "Fast TAQ (v3)".

Set up the following temperature profile:

Hold

95°C 3 min

Cycling – 50 cycles

95°C 5 s

55°C 20 s Acquire on "Green" and "Yellow"

72°C 10 s

Hold after cycling

40°C 60 s

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_MIC_v00.mictemplate". The Run Template can be copied from the CD included in the kit.

Reagent preparation

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mix on vortex and shortly spin.
- In the step of Proteinase K addition of Isolation protocol add 20 µl of Internal Control DNA 01 (IC DNA 01) to isolated sample. In no case add the internal control to isolated DNA just before the analysis.
- **If you do not use all the volume of MasterMix, store the tube at dark at temperature 4°C up to 14 days. For long-term storage use the freezer (-20 °C, dark). MasterMix should not go through more than five freeze- thaw cycles.**

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.



Preparation of Reaction Mix for the instruments LightCycler 480, Rotor-Gene, CFX96 and MIC qPCR

1. Detection: Take one microtube with HSV1 UNI Mix and HSV2 UNI Mix. After the thawing pipette 15 µl of the Mix to amplification microtube or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples.
2. Quantification Standards: Take one microtube with HSV1 UNI Mix and HSV2 UNI Mix and after the thawing pipette 15 µl of MasterMix to microtubes or plate and add 5 µl of the quantification standard of given concentration. Thaw the standard perfectly and warm up to room temperature (warm it in your hand), after thawing, vortex the standard properly and spin on centrifuge shortly. Repeat this procedure with all other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

Preparation of Reaction Mix for LightCycler® 2.0

1. Preparation of MasterMix: Prepare the MasterMix by mixing EliBlocker solution with HSV1 UNI Mix and HSV2 UNI Mix. Take one tube of HSV1 UNI Mix and one with HSV2 UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtubes and to each microtube with HSV1 and HSV2 UNI Mix add 3.0 µl of EliBlocker. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with HSV1 and HSV2 MasterMix and pipette 15 µl of each Mix to glass capillaries and add 5 µl of isolated DNA to HSV1 and HSV2 MasterMix. During the pipetting of samples be careful to avoid cross-contamination of samples.
3. Quantification Standards: Take one microtube with HSV1 and HSV2 MasterMix and pipette 15 µl of each Mix to glass capillaries and add 5 µl of the quantification standard of given concentration. Thaw the standard perfectly and warm up to room temperature (warm it in your hand), after thawing, vortex the standard properly and spin on centrifuge shortly. Repeat this procedure with all other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

Preparation of Reaction Mix for instruments QuantStudio 3 and 5 and ABI7500

1. Preparation of MasterMix: Prepare the MasterMix by mixing ROX dye solution with HSV1 UNI Mix and HSV2 UNI Mix. Take one tube of HSV1 UNI Mix and one with HSV2 UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtubes and to each microtube with HSV1 and HSV2 UNI Mix add 3.0 µl of ROX dye solution. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with HSV1 and HSV2 MasterMix and pipette 15 µl of each Mix to amplification microtubes or plate and add 5 µl of isolated DNA to HSV1 and HSV2 MasterMix. During the pipetting of samples be careful to avoid cross-contamination of samples.
3. Quantification Standards: Take one microtube with HSV1 and HSV2 MasterMix and pipette 15 µl of each Mix to amplification microtubes or plate and add 5 µl of the quantification standard of given concentration. Thaw the standard perfectly and warm up to room temperature (warm it in your hand), after thawing, vortex the standard properly and spin on centrifuge shortly. Repeat this procedure with all other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.



Result reading

LightCycler® 2.0 (Roche):

Analyze real-time PCR results in channels 530 and 560 (LightCycler® Software 4.X) respectively, using the Absolute quantification module of the LightCycler® Analysis Software. Enter the concentrations of HSV1/HSV2 Quantification Standards in FAM channel that are mentioned on single microtubes.

Positive result for *HSV1*: The positive result is characterized by amplification and growth of signal in FAM channel (530) of HSV1 UNI Mix. In a case of negative results the amplification will not occur.

Positive result for *HSV2*: The positive result is characterized by amplification and growth of signal in FAM channel (530) of HSV2 UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (560) of both HSV1 UNI Mix and HSV2 UNI Mix.

Cp value corresponds to the quantity of positive result. The value of "Score" must be higher than 4.5.

LightCycler® 480 (Roche):

In "Sample Editor" menu choose "Abs Quant" workflow. Enter the concentrations of HSV1/HSV2 Quantification Standards in FAM channel that are mentioned on single microtubes.

In menu "Analysis" choose "Abs Quant/2nd Derivative Max" option.

In Analysis window click to "Color Comp" icon and choose Universal CC FAM (510)-VIC (580) calibration. Analyze results by clicking to icon "Calculate".

Positive result for *HSV1*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of HSV1 UNI Mix. In a case of negative results the amplification will not occur.

Positive result for *HSV2*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of HSV2 UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (533-580) of both, HSV1 UNI Mix and HSV2 UNI Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result.

LightCycler® Nano (Roche):

In "Samples" enter the concentrations of HSV1/HSV2 Quantification Standards in FAM channel that are mentioned on single microtubes.

In "Analysis" menu click in window "Select Analysis" on icon "+" and choose "Automatic Quantification".

HSV1/HSV2 analysis

For HSV1 and HSV2 analysis enter in "Settings" for FAM channel following values:

Excluded early cycles: 3; Min. Relative Amp: 0.1; Min. Amp. Quality: 5

Positive result for HSV1: The positive results are characterized by amplification and growth of signal in FAM channel (510-528) of HSV1 UNI Mix. In a case of negative results the amplification will not occur.

Positive result for HSV2: The positive results are characterized by amplification and growth of signal in FAM channel (510-528) of HSV2 UNI Mix. In a case of negative results the amplification will not occur.

Internal control Analysis

For Internal Control analysis enter in "Settings" for HEX channel (530-548) following values:

Excluded early cycles: 3; Min. Relative Amp: 0.1; Min. Amp. Quality: 1

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (530-548) of both, HSV1 UNI Mix and HSV2 UNI Mix.



The values of Concentration correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

RealTime System ABI7500 (Applied Biosystems):

In menu “Setup” enter the concentrations of HSV1/HSV2 Quantification Standards in FAM channel that are mentioned on single microtubes in the software of RealTime instrument.

In “Analysis Settings” choose “Automatic Threshold” and “Automatic Baseline” option and analyze results.

Positive result for HSV1: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm) of HSV1 UNI Mix. In a case of negative results the amplification will not occur.

Positive result for HSV2: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm) of HSV2 UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (em. 548 nm) of both, HSV1 UNI Mix and HSV2 UNI Mix.

The values of Qty correspond to the quantity of positive result, “result undet.” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

Rotor-Gene 6000 or Q (Qiagen)- version 1.7 and higher:

Click to “Edit Samples” icon in the menu and choose for Quantitation Standards Type “Standard” and for samples Type “Unknown”. Enter the concentrations of HSV1/HSV2 Quantification Standards that are mentioned on single microtubes.

Click to “Analysis” icon in the menu and choose Analysis option “Quantitation”. In “Quantitation Analysis” window choose “Dynamic Tube” and “Slope Correct” option.

For HSV1 and HSV2 analysis (Green channel) use Threshold value of 0.1 and for internal control analysis (Yellow channel) use Threshold value of 0.05.

Positive result for HSV1: The positive result is characterized by amplification and growth of signal in FAM channel (Green) of HSV1 UNI Mix. In a case of negative results the amplification will not occur.

Positive result for HSV2: The positive result is characterized by amplification and growth of signal in FAM channel (Green) of HSV2 UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (Yellow) of both HSV1 UNI Mix and HSV2 UNI Mix.

The values of “Calc. conc.” correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

QuantStudio 3 and 5 (ThermoFisher Scientific):

In “Analyse Settings” choose “Automatic Threshold” and “Automatic Baseline” option and analyze results.

Positive result for HSV: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX/VIC channel (em. 548 nm).



CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Data Analysis window choose "Quantification". In "Settings" menu choose option "Baseline Threshold" and select "Baseline Cycles" option as "Auto Calculated" and Single "Threshold" option as "Auto Calculated".

In Data Analysis window select a single fluorophore (FAM or HEX) by the clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

Positive result for HSV: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX channel (em. 548 nm).

MIC qPCR (Bio Molecular Systems):

To view and analyze acquired data, click the small cross next to Cycling under Analysis module and then select the target – Non-Assay Green (FAM) and Non-Assay Yellow (HEX). In the down right corner **increase "Fluorescence Cut-off Level" to 20 %**. A quantification cycle (Cq) value is displayed for each positive well. This is the cycle number at which the sample fluorescence rises above the threshold (the red horizontal line near the bottom of the amplification curve chart). In the Samples panel on the right, click sample names to hide or display the associated amplification curves and result data.

Positive result for HSV: The positive result is characterized by the growth of fluorescence signal in Green channel. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in Yellow channel.

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM channel of both, HSV1 UNI Mix and HSV2 UNI Mix, does not appear before cycle number 45, the result of test should be interpreted as probably HSV1 and HSV2 DNA negative or with concentration of HSV1 and HSV2 DNA below the detection limit of this kit (10 genomic DNA/reaction). The signal for Internal Control must be positive. This result does not exclude the occurrence of HSV1 and HSV2 infection because result of this test depends on proper sample collection and elaboration. As well result depends on enough quantity of analyzed HSV1 and HSV2 DNA.

Positive result:

HSV1

Amplification signal in FAM channel of HSV1 UNI Mix will appear before cycle number 45. HSV1 DNA was detected in the sample. The sample is HSV1 DNA positive.

HSV2

Amplification signal in FAM channel of HSV2 UNI Mix will appear before cycle number 45. HSV2 DNA was detected in the sample. The sample is HSV2 DNA positive.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Inhibited sample:

In the case that increasing of amplification signal in FAM channel (specific for HSV1 and HSV2) as well as increasing of amplification signal in HEX channel (specific for Internal Control) is not observed, it is necessary to repeat the analysis. The best would be to use new DNA prepared by new extraction process.



Control procedure

EliGene[®] HSV1/HSV2 UNI Kit involves Internal Control and Quantification Standards. Internal isolation control follows the quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is HSV1 or HSV2 DNA negative, the Cp of internal control must be less than 35. In the case of strongly positive samples usually the internal control amplification is not detected.

Positive control follows the proper function of MasterMix. Minimal Cp of positive control must be 35 or less. The Cp higher than 35 for Positive Control can't be accepted and DNA detection must be repeated with new sample. In the case of repeatedly higher Cp contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.

Usage the HSV1/HSV2 Quantification Standards:

To generate a standard curve on Instruments, all 4 quantitation standards should be used and defined in the "Edit Samples" dialog box as standards in FAM channel with the specified concentrations (see the instrument user manual).

Applied Biosystems RealTime System 7500, QauntStudio 3 and 5, CFX96, MIC qPCR and RotorGene 6000 or RotorGene Q (Qiagen) instruments will perform the correlation coefficient calculation of standard curve labeled r by entering the appropriate values of calibrators. The value r of the correlation coefficient of standard curve must be higher than 0.9.

LightCycler[®] 480 and 2.0 instruments will perform the standard error calculation of standard curve labeled "Error" by entering the appropriate values of calibrators. The value "Error" of standard curve must be lower than 0.1.

In this case, the device managed on the basis of the measured results of calibrators, build usable calibration line, whereby precisely subtract the results of other analyzed samples. Otherwise, it is necessary to repeat the analysis. Insufficient value of correlation coefficient or error can be caused by bad pipetting, insufficient vortexing of thawed calibrators or inappropriate storage of calibrators.

Reference material:

To monitor the all examination process covering DNA isolation and RealTime PCR detection is possible to use reference viral material. The positive material is possible to order from the Acrometrix company (Life Technologies).

Troubleshooting:

1. If there is no amplification of Internal Control DNA 01 (IC DNA 01), there is some problem in the isolation of DNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Quantification Standards, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

Kit EliGene[®] HSV1/HSV2 UNI has a very high sensitivity - detects 10 genomic DNA added to the amplification mix. The sensitivity of test was verified as follows.

A specific-cloned insert of DNA (required concentration) was prepared and diluted to get desired concentrations of target sequence.

Analytical sensitivity is 10 copies of HSV1 and HSV2 DNA in reaction mix.

Analytical specificity of method is 100%. Specificity of the methods was validated by searching the DNA databases and by addition of human DNA to mastermix. 50 different samples of human DNA did not give false



positive result. Moreover, addition of DNA from *M. tuberculosis*, *M. cansasii*, *M. xenopii*, *M. avium* and *M. marinum*, *C. trachomatis*, *E. coli*, *A. niger*, *C. albicans*, ADV, HBV, EBV, CMV, VZV, HCV, did not give false positive result too.

Clinical specificity was tested on human blood and serum samples. 50 samples of different human DNA isolated from the blood and serum did not give false positive result.

Diagnostic performance characteristics:

Within the frame of testing the functional characteristics of EliGene® HSV1/HSV2 UNI kit overall 636 HSV1 clinical specimens and 211 HSV2 clinical specimens were analyzed. From 636 HSV1 clinical specimens were 201 positive. EliGene® HSV1/HSV2 UNI kit analyzed 197 samples as positive. Totally 435 clinical specimens were properly by EliGene® HSV1/HSV2 UNI kit analyzed as negative. From 211 HSV2 clinical specimens were 24 positive. EliGene® HSV2 UNI kit analyzed all 24 samples as positive. Totally 187 clinical specimens were properly by EliGene® HSV2 UNI kit analyzed as negative.

HSV1 Sensitivity: 98.04%

HSV1 Specificity: 100%

HSV2 Sensitivity: 100%

HSV2 Specificity: 100%

Measuring interval

The kit enables the detection of 10^1 - 10^8 of viral DNA molecules in reaction mix.

Internal control of quality

As an internal control of quality the Internal Control for checking the process of DNA isolation together with Quantification Standards for functional control of MasterMix and as a reference sample is used.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of DNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Biological reference intervals

Not applicable information for this kit.

Warning

Unused content of the tube with MasterMix is stable for 2 weeks at -20 °C. Do not freeze tubes with MasterMix repeatedly! Do not mix components of the kits of different lots.

Warnings and general precautions

This kit is intended for *in vitro* use only.

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and



disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.

- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer's kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

- The tubes containing mixes (HSV1 UNI Mix, HSV2 UNI Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC DNA 01 are disposable and therefore must be used once only in the preparation of the reaction mixture.
- These mixes carry the following safety warnings (P):



P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

Literature

Barbara A. Bannister, Norman T. Begg and Stephen H. Gillespie: Infectious Disease. Blackwell Science, 2th Ed., 2000

Hlinomazová Z, Loukotová V, Horáčková M, Šerý O. 2010. The treatment of HSV1 ocular infections using quantitative real-time PCR results. Acta Ophthalmol. 10: 1755-3768

Symbols



Catalog number



Upper limit of temperature



Batch code



Use by (last day of month)



in vitro diagnostic medical device



Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.



Contains sufficient for "N" tests



Attention, consult instructions for use



Manufacturer

Manufacturer

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