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EliGene®Enterovirus LC

REF 90053-LC (for 50 samples)

Kit components:

10 x 75 µl **Entero LC Mix**
1 x 55 µl **Enzyme Mix**
5 x 100 µl **IC RNA**
2 x 50 µl **PC DNA Entero**
1 x 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

EliGene® Enterovirus LC is intended for the detection and the quantification of *Enterovirus* RNA.

Principle of the method

This diagnostic kit is based on Reverse Transcription quantitative PCR method in one tube. In this kit primers and labeled probes (FAM and HEX) for the detection of *Enterovirus* RNA and for the detection of internal control are used.

Introduction

Human Enterovirus is a genus of (+)ssRNA viruses associated with several human and mammalian diseases.

Until now, 66 serotypes are known of which there are 62 non-polio Enteroviruses that can cause disease in humans: 23 *Coxsackie* A viruses, 6 *Coxsackie* B viruses, 28 echoviruses, and 5 other Enteroviruses. Enteroviruses are ubiquitous pathogens with a high incidence worldwide (ca. 500 million infections/year) and are often found in the respiratory secretions (e.g., saliva, sputum, or nasal mucus) and stool of an infected person. Enteroviruses may cause life-threatening infections, especially among children when diseases such as myocarditis, paralysis, multiple organ failure, meningitis and encephalitis may be associated with infections.

Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
CSF, serum, plasma	Manual: Chemagic Viral DNA/RNA Kit (Chemagen-PerkinElmer)
	Automatic: **ZEPHYRUS Magneto** (ELISABETH PHARMACON)

Serum or plasma: According to standard protocol, take the sample of serum or plasma into sterile tubes. Samples must be stored and transported at -20 °C.

The kit is optimized for usage of isolation kit Chemagic Viral DNA/RNA according to standard protocol and MAGNETO ***BodyFluid DNA/RNA isolation kit*** according to standard protocol for plasma samples with elution to 50 µl of PCR water.

Start volume is 200 µl of serum or plasma with elution to 50 µl of PCR water. Before the isolation 10 µl of Internal Control RNA (IC RNA) must be added to the sample. It is highly recommended isolate RNA to PCR

water because inhibition due to composition of elution buffer can occur.

It is highly recommended use RNA immediately after the isolation for the analysis. You can store RNA at -20 °C but concentration of isolated RNA is getting lower in every freezing process and there is a risk of obtaining of false negative results. That is the reason why to carry out RT-PCR immediately after the RNA isolation.

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® 480, QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific), Rotor-Gene Q (Qiagen) and CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The RT-qPCR for the detection of Enterovirus RNA utilizes TaqMan technology (FAM and HEX probes) and can be performed on other instruments that can work in FAM and HEX channels.
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

- For Enterovirus detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm)
- For Internal Control the probe labeled with HEX is used (exc. 520 nm – em. 548nm)

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

45°C	15 min	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 2 - Analysis mode “None”, 1 Cycle

95°C	2 min	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 2 - Analysis mode “Quantification”, 50 Cycles

95°C	15 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	25 s	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 1 - Analysis mode “None”, 1 Cycle

40°C	20 s	Ramp rate (2.2°C/s)	Acquisition mode “None”

The complete temperature profile can be up-loaded from Run Template “Entero_LC480_v02.ixo”. The Run Template can be imported to the software in menu “Navigator” by clicking to icon “Import” from the CD included in the kit.

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QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

Use the Experiment type,"Presence/Absence", Chemistry "TaqMan Probes", and Run Mode "Standard".

Set up the following temperature profile:

Holding stage

45°C 15 min Ramp rate (1.6°C/s)

Holding stage

95°C 2 min Ramp rate (1.6°C/s)

Cycling stage – 50 cycles

95°C 15 s Ramp rate (1.6°C/s)

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

72°C 25 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 20 s Ramp rate (1.6°C/s)

Collect emission signal at the second step of cycling stage at 55 °C

The complete temperature profile can be up-loaded from Run Template "Entero_QS3_v00.edt" or "Entero_QS5_v00.edt.edt". The Run Template can be copied from the CD included in the kit.

Rotor-Gene 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

45°C 15 min

Holding stage

95°C 2 min

Cycling stage – 50 cycles

95°C 15 s

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

72°C 25 s

Holding stage

40°C 20 s

For the Gain optimization in all channels select option "Automatic gain optimization before first acquisition". The complete temperature profile can be up-loaded from Run Template "Entero_Q-GENE_v02.ret". 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	45°C	15 min
Step 2	95°C	2 min
Step 3	95°C	15 s
Step 4	55°C	20 s + Plate Read
Step 5	67°C	25 s
Step 6	*GOTO Step 3*	*49x*
Step 7	40°C	20 s

Enter the Sample Volume 20ul

Collect emission signal at the Step 4 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 (Samples) or Standard". The complete temperature profile can be up-loaded from Run Template "Entero_CFX_v00.prcl". The Run Template can be copied from the CD included in the kit.

If using instruments other than the validated ones listed in this manual, the parameters for reading and analyzing the results must be set for such instrument. These settings must be verified by comparison with the results obtained on one of the validated instruments. For comparison purposes, reference samples or samples from external quality assessment can be used. If the parameters for reading and analysis of the results are not verified, the criteria for interpretation of the results given in the manual cannot be applied to non-validated instruments, as incorrect results may be given. In these cases, the manufacturer does not bear responsibility for any issues related to incorrect result interpretation.

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 10 µl of Internal Control RNA (IC RNA) to isolated sample. In no case add the internal control to isolated RNA just before the analysis.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for Master Mixes, separate pipette for positive controls and separate pipette for samples! Follow all recommendations for laboratories providing RNA analyses.

Preparation of Reaction Mix

1. Preparation of MasterMix: Prepare the MasterMix by mixing Enzyme Mix with Entero LC Mix. Take one tube of Entero LC Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtube and add 5 µl of Enzyme Mix. Mix gently by pipetting up and down and shortly spin.
2. Detection: Add 15 µl of the Master Mix to the amplification tubes or plates and add 5 µl of the isolated RNA sample. Be careful when pipetting the sample to avoid cross-contamination of the samples. The

prepared Master Mix should be used within 30 minutes and cannot be reused. Do not freeze prepared Master Mix.

3. Positive Control: Take one microtube with MasterMix and pipette 15 µl of MasterMix to the glass capillary and add 5 µl of PC DNA Entero. During the pipetting of Positive Control be careful to avoid contamination of other samples. Use separate pipette for positive controls.

Insert the microtubes or plate into the RealTime PCR instrument and run the program as described in Configuring the RealTime PCR Instrument above.

Result viewing

LightCycler® 480 (Roche):

In "Sample Editor" menu choose "Abs Quant" workflow.

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

In Analysis window choose from "ColorComp" menu "In Database" and "Universal CC FAM (510)-VIC (580)" Color Compensation.

Positive result for Enterovirus: The positive result is characterized by the growth of fluorescence signal in FAM channel (465-510). 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 a growth of signal in HEX channel (533-580).

QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

In "Analyse Settings" choose "Automatic Treshold" and "Automatic Baseline" option and analyze results.

Positive result for Enterovirus: 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).

Rotor-Gene Q (Qiagen):

Click to "Analysis" icon in the menu and choose Analysis option "Quantitation". In "Quantitation Analysis" window choose "Dynamic Tube" and "Slope Correct" option. In "Outlier Removal" option set-up NTC Treshold of 10 %.

Positive result for Enterovirus: The positive result is characterized by the growth of fluorescence signal in FAM channel (Green). 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 (Yellow).

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

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 Enterovirus: 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).

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM channel does not appear before cycle number 50, the result of test should be interpreted as probably *Enterovirus* RNA negative or with concentration of *Enterovirus* RNA below the detection limit of this kit (10 genomic RNA/reaction). The signal for Internal Control (IC RNA) must be positive. This result does not exclude the occurrence of Enterovirus infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed *Enterovirus* RNA.

Positive result:

The amplification signal in FAM channel will appear before cycle number 45. *Enterovirus* RNA was detected in the sample. The sample is *Enterovirus* RNA 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 RNA and DNA analyses.

Inhibited sample:

In the case that increasing of amplification signal in FAM channel (specific for *Enterovirus*) is not observed and Ct value in HEX channel (specific for Internal Control) is higher than 30, it is necessary to repeat the analysis. The best would be to use RNA prepared by new extraction process.

Control procedure

EliGene® Enterovirus LC Kit involves Internal Control (IC RNA). To control the production of the mastermix, the internal control at a very low concentration giving (Ct > 34) is involved in the reaction mix. To control isolation yield and occurrence of an inhibition of amplification process the Internal Control (IC RNA) must be added before the RNA isolation directly to the sample, and in the case that the sample is *Enterovirus* RNA negative, Ct of Internal Control must be Ct < 30.

Reference material:

To monitor the all examination process covering RNA 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, there is some problem in the isolation of RNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

The sensitivity of EliGene® Enterovirus LC Kit is 600 genomic RNA (Enteroviruses) in 1 ml of serum and it depends on the method of the isolation procedure. The sensitivity of method was verified as follows. There were used the RNA samples of known concentration of genomic RNA. Totally it was tested for three times. The *Enterovirus* detection was 100% successful in all the samples which contain 600 and more genomic RNA/ml serum.

Analytical sensitivity is 10 copies of *Enterovirus* RNA in reaction mix.

Analytical specificity of method is 100%. Analytical specificity of method was analyzed by comparison of primers and probes with all known RNA and DNA sequences in GenBank database. Analytical specificity was also analyzed by the addition of DNA from EBV, CMV, HSV1, HSV2, VZV, MTB, *Borrelia* sp., *C. trachomatis*, *E. coli*, *A. niger*, *C. albicans* to the reaction mix. These DNA or RNA did not give false positive result for Enterovirus.

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

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 (IC RNA) for checking the process of RNA isolation together with Positive Control 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 RNA). It is strictly recommended to use isolation kits and procedures mentioned above.

The sensitivity of the detection of *Enterovirus* RNA is dependent on proper sample collection, storage and elaboration (isolation of RNA, the date of receipt of the sample, detection immediately after isolation).

Biological reference intervals

Not applicable information for this kit.

Warning

After mixing, MasterMix is stable for 30 minutes. Do not freeze tubes with MasterMix repeatedly! Do not mix components of the kits of different lots.

Warnings and general precautions

- 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 flow hood. Tubes containing different samples must never be 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 a laminar flow hood. 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 a 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 Entero LC Mix is disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC RNA 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.

In the case of any problems contact our customer support ELISABETH PHARACON, spol. s r. o.

Literature

Josko D. Molecular virology in the clinical laboratory. Clin Lab Sci. 2010 Fall;23(4):231-6.
Bannister BA, Begg NT, Gillespie SH. 2000. Infectious Disease. Blackwell Science, 2th Ed.

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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