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EliGene® HBV RT

REF **90037-RT (for 50 samples)**

Kit components:

5 x 200 µl **HBV Mix**
5 x 200 µl **IC DNA 01**
1 x 100 µl **HBV QS1 (1 x 10^1 IU/µl)**
1 x 100 µl **HBV QS2 (1 x 10^2 IU/µl)**
1 x 100 µl **HBV QS3 (1 x 10^3 IU/µl)**
1 x 100 µl **HBV QS4 (1 x 10^4 IU/µl)**
1 x 100 µl **HBV QS5 (1 x 10^5 IU/µl)**
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® HBV RT is intended for the detection and the quantification of Hepatitis B virus DNA.

Principle *of the method*

This kit is based on RealTime PCR method. In this kit primers and labeled probes (FAM and HEX) for the detection of HBV DNA and for the detection of internal control are used.

Introduction

Hepatitis B virus is a small DNA virus divided into *Hepadnaviridae* family. Eight different genotypes signed by alphabetical letters A to H, have been previously described. Since the proposal of the four described genotypes (A–D), four others (E–H) have been characterized during the two last decades. Recently, a ninth genotype evidenced in North-West China, India, Lao and Vietnam and tentatively termed “I” was suggested, although it is still subject to debate and as being a recombinant strain with a genotype C backbone. Finally, very recently, a tenth genotype provisionally assigned to genotype “J” was proposed for a Japanese patient's HBV isolate.

Hepatitis B (HB) is transmitted by the exchange of body fluids e.g. blood, semen, breast milk and saliva. The virus spread between people who have unprotected sexual intercourse, drug users who share needles and syringes and health care workers in contact with potentially contaminated blood or body fluids.

It is estimated that one third of the world's population has serological evidence of past or present infection with HBV and that 350 to 400 million people are still chronically infected, of whom 78 % lived in Asia, 16 % in Africa, 3 % in South America and the remaining 3% in Europe, North America and Oceania.

HBV infection has a broad spectrum of clinical diseases, ranging from acute hepatitis (including fulminant hepatic failure) to a low viraemic asymptomatic “inactive” carrier state or to progressive chronic hepatitis that may lead to cirrhosis with an annual rate of 2 to 5 % in HBe-positive patients and hepatocellular carcinoma (HCC) with a cumulative 5-year incidence of 15 to 20 %. Both HBV-related end-stage liver disease and HCC are responsible for around 1 million deaths per year.

Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Serum, plasma	Manual: Chemagic Viral DNA/RNA Kit (Chemagen-PerkinElmer)
	Automatic: Prepito Viral DNA/RNA1k Kit (Chemagen-PerkinElmer)

WARNING: To keep the sensitivity of the test we recommend follow strictly the pre-analytical procedures mentioned in this instruction. Specially do not change the recommended procedures of isolation as for stated amount of specimen. For DNA isolation other DNA isolation kits can be used but pre-analytical procedures for storage and transport of samples must be followed. During isolation from smaller quantity of sample it is necessary to make elution into adequate volume of elution buffer for keeping of method sensitivity. E.g. in case of isolation from 200 microliters of sample to 50 microliters of elution buffer, the sensitivity of the method would be 5 times lower.

Serum, plasma:

According to standard protocol take the sample of the serum or plasma into sterile tubes (EDTA). Samples must be stored and transported at 4 °C. It is recommended to isolate DNA from the sample during the day of taking sample or store at -20°C maximally for one month. For DNA isolation at least 200 µl of sample must be used.

Manual isolation:

Add 20 µl of Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01) to 200 µl of the specimen and then continue according to the standard protocol of Chemagic Viral DNA/RNA Kit with final elution step in 25 µl of Elution buffer. 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 20 µl of Internal Control DNA 01 (IC DNA 01) to 1.0 ml of the specimen and isolate DNA from the sample by using Prepito Viral DNA/RNA1k Kit according to protocol for plasma samples with elution in 50 µl of Elution buffer. In the case of smaller quantity of the serum specimen fill up specimen to 1.0 ml by molecular grade water

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 ABI 7000, 7300, 7500 (Applied Biosystems), LightCycler 480 and LightCycler Nano (Roche), RotorGene 6000 or RotorGene Q (Qiagen), CFX96 Touch Real-Time PCR Detection System (Bio-Rad), MIC qPCR Cycler (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 HBV detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm).
- For Internal Control DNA 01 the probe labeled with HEX is used (exc. 520 nm – em. 548 nm).
- Reaction Mix includes passive reference control dye ROX for signal normalization.

RealTime Systems ABI 7000, 7300, 7500FAST (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 ABI7500FAST use "7500 (96wels)" instrument type.

Set up the following temperature profile:

Holding stage

95°C 3 min

Cycling stage – 50 cycles

95°C 15 s

60°C 60 s Data collection ON

Collect emission signal at the second step – 60 °C.

The complete temperature profile can be up-loaded from Run Template "HBV_RT_ABI7300_v00.sdt" or "HBV_RT_ABI7500_v00.edt". The Run Template can be copied 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	15 s	Ramp rate (4.4°C/s)	Acquisition mode "None"
60°C	60 s	Ramp rate (2.2°C/s)	Acquisition mode "Single"

Step 1 - 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 "HBV_RT_LC480_v00.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.

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 – 2-Step Amplification, 50 cycles

95°C	15 s	Ramp rate (5°C/s)	
60°C	60 s	Ramp rate (4°C/s)	"Acquire" signal

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 “HBV_RT_LCNANO_v00.ppf”. 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	15 s
Step 3	60°C	60 s + Plate Read
Step 4	*GOTO Step 2*	*50x*
Step 5	40°C	20 s

Enter the Sample Volume 30 µl

Collect emission signal at the Step 3 at 60° 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”.

MIC qPCR (Bio Molecular Systems Pty Ltd):

In Run Setup Create a new Run Profile for the MIC instrument. Enter the Sample Volume of 30 µl and Temperature Control “Fast TAQ (v3)”.

Set up the following temperature profile:

Hold

95°C 3 min

Cycling – 50 cycles

95°C 15 s

60°C 60 s Acquire on "Green" and "Yellow"

RotorGene 6000 or Q (Qiagen):

In the “New Run” window choose “Two 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 15 s

60°C 60 s Acquiring in channels “Green” and “Yellow”

The complete temperature profile can be up-loaded from Run Template “HBV_RT_RG6000_v00.ret”. 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.
- Isolate DNA according to standard protocol.

Preparation of Reaction Mix

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.

1. The detection: Take one microtube with HBV Mix and after the thawing pipette 20 µl of the Mix to amplification microtube or plate and add 10 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples. If you do not use all the volume of HBV Mix, freeze it and store at -20 °C in a dark. Do not freeze tubes with HBV Mix repeatedly. Under these conditions it is stable at least for 14 days.
2. Quantification Standards: Take one tube with HBV Mix and after the thawing pipette 20 µl of the Mix to the amplification microtube or plate and add 10 µl of the 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 microtubes or plate with samples to the RealTime PCR instrument and run the program according to chapter “Configuration of Real Time instrument” above.

Result viewing

RealTime Systems ABI 7000, 7300, 7500FAST (Applied Biosystems):

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

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

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm). 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).

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.

LightCycler® 480 (Roche):

In “Sample Editor” menu choose “Abs Quant” workflow. Enter the concentrations of HBV 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: The positive results are characterized by amplification and growth of signal in FAM channel (465-510). 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).

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.

LightCycler® Nano (Roche):

In “Samples” enter the concentrations of HBV Standards in FAM channel that are mentioned on single microtubes.

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

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (510-528). 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 (530-548).

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.

RotorGene 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 HBV 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.

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (Green). 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).

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.

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: 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: 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 does not appear before cycle number 50, the result of test should be interpreted as probably HBV DNA negative or with concentration of HBV DNA below the detection limit of this kit (0.1 IU/µl of the isolated sample). The signal for Internal Control must be positive. This result does not exclude the occurrence of HBV infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed HBV DNA.

Positive result:

Amplification signal in FAM channel appears before cycle number 50. HBV DNA was detected in the sample. The sample is HBV 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 HBV) 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® HBV RT Kit involves Internal Control DNA 01 (IC DNA 01) and Quantification Standards (QS1-5). Internal Control follows the quality of DNA isolation and detects the occurrence of an inhibition of amplification process. In the case that the sample is HBV DNA negative, the Cp of internal control must be Cp < 35. In the case of strongly positive samples usually the internal control amplification is not detected.

Quantification Standards follow the proper function of MasterMix. Minimal Cp of the lowest HBV quantification standard (HBV QS1) must be 35 or less. The Cp higher than 35 can't be accepted and analysis must be repeated.

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 HBV quantification standards:

To generate a standard curve on Instruments, all 5 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 7000, 7300, 7500 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 vaule r of the correlation coefficient of standard curve must be higher than 0.9.

LightCycler® 480 instrument 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.

LightCycler® 480 instrument can perform evaluation by using external calibration curve. We recommend a new calibration for each new shipment or with each new kit lot.

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.

The Quantitation Standards are defined as IU/µl. The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material:

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/µl)} \times \text{Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

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, 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 Standards, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

The Limit of detection (LOD) in consideration of the purification (Prepito Viral DNA/RNA1k Kit) of the EliGene® HBV RT Kit was determined using a dilution series of the HBV Specimen from 100 to nominal 0.5 IU/ml HBV spiked in HBV negative clinical plasma specimen. These were subjected to DNA extraction using the Prepito Viral DNA/RNA1k Kit (extraction volume: 1.0 ml, elution volume: 50 µl). Each of the five dilutions was analysed by the ABI7300 instrument (Life Technologies, USA) with the EliGene® HBV RT Kit on three replicates. The analytical detection limit in consideration of the purification of the EliGene® HBV RT Kit is 5 IU/ml for Prepito Viral DNA/RNA1k Kit. This means that 100% sensitivity can be declared for the kit for the concentration of 5 IU/ml of serum. After recalculation to isolated specimen, the sensitivity of tested kits is equal to concentration of 0.1 IU/µl of isolated specimen.

Analytical sensitivity is 0.1 IU/µl of isolated specimen.

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

Control group	HBV (FAM)	IC DNA 01 (HEX)
Human herpesvirus 1 (Herpes simplex virus 1)	-	+
Human herpesvirus 2 (Herpes simplex virus 2)	-	+
Human herpesvirus 3 (Varicella-zoster virus)	-	+
Human herpesvirus 4 (Epstein-Barr virus)	-	+
Human herpesvirus 5 (Cytomegalovirus)	-	+
Mycobacterium tuberculosis	-	+
Borrelia sp.	-	+
Chlamydia trachomatis	-	+
Escherichia coli	-	+
Aspergillus niger	-	+
Candida albicans	-	+

Clinical specificity was tested on negative human serum samples. 200 samples of different human DNA isolated from the serum did not give false positive result.

Performance characteristics:

Within the frame of testing the functional characteristics of EliGene® HBV RT Kit overall 500 clinical specimens were analyzed. From these specimens, 300 HBV positive specimens and 200 HBV negative specimens were confirmed by *artus*® HBV TM PCR Kit. The EliGene® HBV RT Kit diagnosed as HBV positive 299 specimens. 1 specimen from Nigeria was determined by the EliGene® HBV RT Kit as negative.

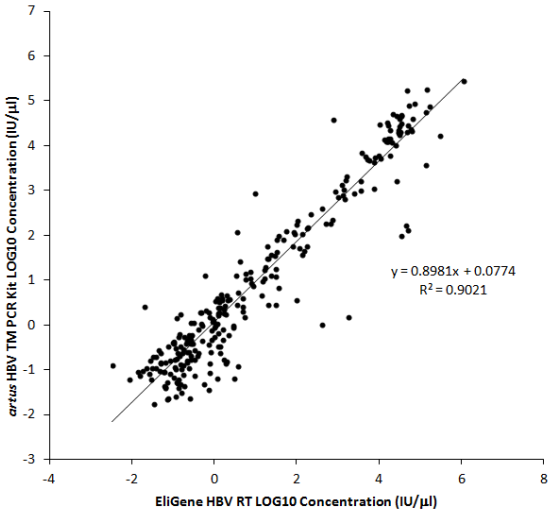
The calculation of the sensitivity and the specificity of the EliGene® HBV RT Kit and the EliGene® HBV UNI Kit are shown in Table below. The sensitivity and specificity of EliGene® HBV RT Kit is 99.7 % and 100 %, respectively.

A = 300 Really positive	B = 0 False positive
C = 1 False negative	D = 200 Really negative

Sensitivity = A/(A+C) = 300/(300+1) = 99,7%

Specificity = D/(D+B) = 200/(200+0) = 100%

The correlation of the quantitative results of EliGene® HBV RT Kit and *artus*® HBV TM PCR Kit was analysed by linear regression. Subsequently, the logarithmised results of both kits were plotted as scatterplots against each other and resulting correlation coefficient was calculated to 0.9.



Comparison of the *artus* HBV TM PCR Kit against the EliGene® HBV RT Kit

Measuring interval

The linear range (analytical measurement) of the EliGene® HBV RT Kit was determined by analysing a dilution series of a HBV quantitation standard ranging from 1 x 10^5 IU/µl to 1 x 10^{-1} IU/µl. Each dilution has been tested in three replicates using the EliGene® HBV RT Kit on the ABI7300 instrument (Life Technologies, USA). The linear range of the EliGene® HBV RT Kit has been determined to cover concentrations from 0.1 IU/µl to at least 1 x 10^5 IU/µl (Figure 1).

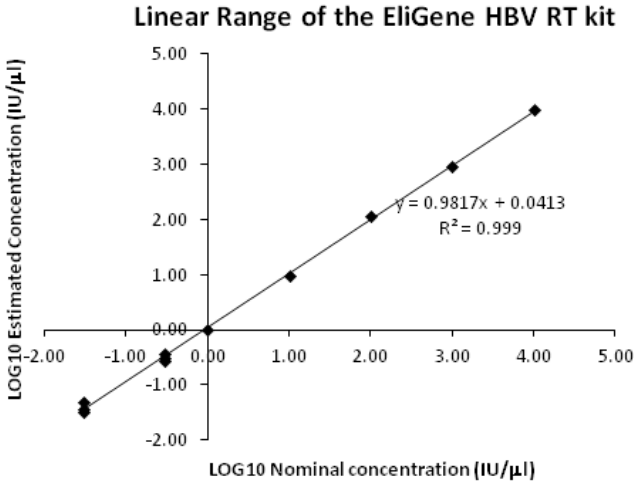


Figure 1. *Calculation of the linear range.* The straight was determined by linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Internal control of quality

As an internal control of quality the Internal Control DNA 01 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 aplicable 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

- 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 mix (HBV 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

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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