184-8 2024-04-22

IVD

For professional use only

HBV PCR detection Kit

USER MANUAL

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REF

R1-P602-23/9EU F1-P602-21/1EU
R1-P602-S3/9EU E1-P602-50/1EU
R1-P602-24/9EU E1-P602-20/1EU
F1-P602-51/1EU

VER 184-8.2024.04.22

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1. INTENDED USE

The **HBV PCR detection Kit** is intended for research and diagnostic applications. The **HBV PCR detection Kit** is an *in vitro* Nucleic Acid Test (NAT) based pathogen detection product. The **HBV PCR detection Kit** is designed to detect Hepatitis B Virus (HBV) nucleic acids in human blood plasma.

The **HBV PCR detection Kit** can be used in clinical practice and for research purposes for HBV diagnostics.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence.

The detection can be performed in each of three variants: real-time (**HBV Real-Time PCR Detection Kit**), endpoint (**HBV FLASH PCR Detection Kit**) and **HBV Conventional PCR Detection Kit**.

The **HBV Real-Time PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes bearing reporter and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided. The **HBV FLASH PCR Detection Kit** is based on the same principle but the fluorescence is measured only once after reaction. **HBV Conventional PCR Detection Kit** is developed for PCR result detection by electrophoresis in the agarose gel.

The automatic analysis available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for HBV Real-Time PCR Detection Kit (see the catalogue at www.dna-technology.ru/en to see available supply options) and Gene or Gene-4 Fluorescence Readers REF O-GENE-EU, O-GENE4-EU for HBV FLASH PCR Detection Kit.

The HBV Real-Time PCR Detection Kit is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers. The HBV FLASH PCR Detection Kit is also approved for use with Ala1/4 fluorescence reader (BioSan).

DNA extraction. On this step the internal control sample (IC) is added to the samples. It is needed for test quality assurance.

3. CONTENT

Table 1. *PREP-NA* DNA/RNA Extraction Kit[1]

Reagent	Description	Total volume	Amount
Lysis buffer	Colorless, soapy liquid	30 mL	1 vial
Precipitation buffer	Colorless liquid	40 mL	1 vial
Washout solution 1	Colorless liquid	50 mL	1 vial
Washout solution 2	Colorless liquid	30 mL	1 vial
Dilution buffer	Colorless liquid	5.0 mL (1.25 mL in each tube)	4 tubes
Negative control (C-)	Colorless liquid	3.0 mL (1.5 mL in each tube)	2 tubes
Internal control (DNA-IC)	Colorless liquid	1.0 mL	1 tube
Internal control (RNA-IC)	Colorless liquid	1.0 mL	1 tube

Table 2. HBV PCR detection Kit

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless liquid and white waxy fractions	1.92 mL or 2.0 mL (0.02 mL per tube)	96 or 100 separate or stripped tubes of 0.2 or 0.5 mL
TECHNO Taq-polymerase	Colorless viscous liquid	50 µL	1 tube
PCR-buffer	Colorless liquid	1 mL (0.5 mL in each tube)	2 tubes
Positive control	Colorless liquid	150 µL	1 tube
Mineral oil (not supplied in Kit for Rotor-Gene Q)	Colorless viscous liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Accessories: Caps for strips	12 pieces[2].		

The approximate total time needed to perform the assay from 4 hours (including sample preparation).

Upon customer's request, optional supply of a reagent kit for DNA electrophoretic detection is possible, including:

- Electrophoresis mix (9.55 g) and Agarose gel (5 plates)

The *PREP-NA* DNA/RNA Extraction Kit is sufficient for extraction of 100 samples.

The **HBV PCR detection Kit** sufficient to test 96/100 samples including negative and positive controls.

[1] - can be included into the kit if requested.
[2] - in case of using stripped tubes.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1 **Specimen collection**

The whole blood samples shoud be collected in 2.0 or 4.0 mL Vacuette type tubes with EDTA in 2.0 mg/mL final concentration. The sodium citrate anticoagulant is also applicable.

 The use of heparin anticoagulant is not allowed.

4.2 **DNA extraction and PCR**

Biological (microbiological) safety cabinet class II;

UV PCR cabinet;

Vortex mixer;

0.2, 0.5 and 1.5 mL tubes;

PCR tube rack for 0.2, 0.5 and 1.5 mL tubes;

Vacuum blood collection tubes (Vacuette for example), containing ethylenediaminetetraacetic acid disodium salt (EDTA) or sodium citrate anticoagulant;

Aspirator with trap flask to remove supernatants;

Single channel pipettes (volume range 0.5-10 µL, 5-40 µL, 40-200 µL, 100-1000 µL);

RNase and DNase free filtered pipette tips (volume range 20 µL, 50 µL, 200 µL, 1000 µL);

Powder-free surgical gloves;

Disinfectant solution;

Container for used pipette tips;

Household refrigerator with a freezer chamber;

High speed centrifuge (RCF 16 000 x g);

Thermostat (temperature range 40-95 °C);

Real-time PCR thermal cycler (for ***HBV*** **Real-Time PCR Detection Kit**);

Tercyc Conventional PCR Thermal Cycler (REF O-TP4-EU) or equivalent (for **HBV FLASH PCR Detection Kit and HBV Conventional PCR Detection Kit**);

Gene or Gene-4 Fluorescence Reader (REF O-GENE-EU, O-GENE4-EU) or Ala1/4 fluorescence reader or equivalent (for **HBV FLASH PCR Detection Kit**);

For electrophoretic detection (for **HBV Conventional PCR Detection Kit**):

- AC power supply;
- electrophoretic chamber;
- transilluminator;
- 1.0 L volumetric flask;
- distilled water;
- 1.0 mm diameter steel wire.

5. WARNINGS AND PRECAUTIONS

The laboratory makeup should comply the requirements regulating work with microorganisms of I-IV classes of pathogenicity.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and cannot be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapour/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Do not use the kit after the expiry date provided. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

6. DNA EXTRACTION PROTOCOL

The **HBV PCR detection Kit** is designed to detect DNA extracted from blood plasma. Shake the tube containing blood sample thoroughly to mix the blood and anticoagulant.

 Using of heparin as anticoagulant is not allowed.

 The overall storage of the sample should not exceed 6 hours.

The transportation and storage temperature from collecting the sample till analysis should be in between 2 °C and 8 °C range.

 Whole blood cannot be frozen.

6.1 To obtain the plasma spin the tubes with blood at 800-1600 x g (corresponds to 3000 rpm on Eppendorf Centrifuge 5424) for 20 min at room temperature (between 18 °C and 25 °C).

 Relative centrifugal force (RCF or g) depends on rotation frequency and centrifugation radius (Annex A). To establish if your centrifuge meets the requirements apply to the exploitation manual for centrifuge.

6.2 Take the upper fraction (plasma) with an automatic sampler and put it into the new 1.5 mL tube. The blood plasma can be stored at temperature from minus 18 °C to minus 22 °C for no longer than 3 months.

 The lysis buffer can contain the precipitate. Dissolve it at 65 °C for 10 min. prior to use.

 At this step of assay use only disposable pipette tips witch have filter and are RNase and DNase free.

6.3 Mark the required number of 1,5 mL tubes by the following scheme: for each test sample and for negative control (C-).

For example: if you need to test 10 samples, mark 11 tubes (10 for the samples, 1 for C-).

6.4 Add 10 µL of the premixed internal control (DNA-IC) in each tube.

6.5 Add 300 µL of the lysis buffer avoiding contact of the pipette tip with an edge of the tube. Close the tubes.

 Open the tube, add sample, then close the tube before proceeding to the next DNA sample to prevent contamination.

6.6 Add 100 µL of the premixed blood plasma sample into the marked tubes. Do not add samples to the “C-“ tube.

6.7 Add 100 µL of the “C-“ into corresponding tube.

6.8 Close the tubes and mix them for 3–5 sec twice and spin down the drops for 3–5 sec at room temperature.

6.9 Incubate the tubes for 15 min at 65 °C, spin down the drops at 16000 x g (corresponds to 13000 rpm on Eppendorf Centrifuge 5424) for 30 sec at room temperature.

6.10 Add 400 µL of the precipitation buffer into all tubes. Close the tubes and mix them for 3-5 sec twice.

6.11 Spin the tubes at 16000 x g (corresponds to 13000 rpm on Eppendorf Centrifuge 5424) for 15 min at room temperature.

6.12 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.13 Add 500 µL of the washout solution №1 to the precipitate and invert the tubes gently 3-5 times.

6.14 Spin the tubes at 16000 x g (corresponds to 13000 rpm on Eppendorf Centrifuge 5424) for 5 min at room temperature.

6.15 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.16 Add 300 µL of the washout solution №2 to the precipitate and invert the tubes gently 3-5 times.

6.17 Spin the tubes at 16000 x g (corresponds to 13000 rpm on Eppendorf Centrifuge 5424) for 5 min at room temperature.

6.18 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.19 Open the tubes and dry the precipitate at 65 °C for 5 min.

6.20 Add 25 µL of the dilution buffer to the precipitate. Spin down the drops for 3–5 sec.

6.21 Incubate the tubes for 10 min at 65 °C.

6.22 Spin down the drops at 16000 x g (corresponds to 13000 rpm on Eppendorf Centrifuge 5424) for 30 sec.

The DNA preparation is ready for PCR amplification.

The DNA preparation can be stored at temperature from minus 18 °C to minus 22 °C for no longer than 1 month or at temperature from minus 68 °C to minus 72 °C for no longer than 1 year.

7. PCR PROTOCOL

7.1 Mark tubes with PCR-mix for each test sample, negative control (C-), positive control (C+). Mark additionally two tubes for background buffer (applicable to FLASH PCR kits).

For example if you need to test 10 samples, mark 12 tubes (10 for samples, 1 for C-, 1 for C+). For FLASH PCR kit mark 14 tubes (10 for samples, 1 for C-, 1 for C+ and 2 for background buffer).

 Mark only the caps of the tubes when using Rotor-Gene Q Thermal Cycler.

7.2 Thaw PCR-buffer at the room temperature.

7.3 Mix the PCR-buffer and TECHNO Taq-polymerase thoroughly (3-5 sec), then spin briefly (1-3 sec) at room temperature.

 Hold TECHNO Taq-polymerase at room temperature as short time as possible. The overheating is detrimental to its performance.

7.4 Prepare the mixture of PCR-buffer and TECHNO Taq-polymerase (TECHNO Taq-polymerase solution). Add into the one tube:

- 10 x (N+1) µL of PCR-buffer;
- 0.5 × (N+1) µL of TECHNO Taq-polymerase;

N — number of the marked tubes including C-, C+, background tubes.

For example if you need to test 10 samples (12 marked tubes), prepare mixture of PCR-buffer and TECHNO Taq-polymerase for 13 (12+1) tubes: 130 µL PCR-buffer + 6.5 µL TECHNO Taq-polymerase.

7.5 Vortex the tube with TECHNO Taq-polymerase solution for 3-5 sec and spin down the drops for 1-3 sec at room temperature. The maximum storage time for prepared mixture at the temperature between 2 °C and 8 °C for no longer than 1 hour.

7.6 Add 10 µL of TECHNO Taq-polymerase solution into each tube (except background tubes). Add 10 µL of background buffer into corresponding tubes (applicable to FLASH PCR kits). Avoid paraffin layer break.

7.7 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close tubes tightly.

7.8 Vortex the tubes with samples for 3-5 sec and spin down the drops for 1-3 sec.

7.9 Vortex the tubes with DNA for 3-5 sec and spin down the drops for 1-3 sec at room temperature.

7.10 Add 5.0 µL of DNA sample into corresponding tube. Avoid paraffin layer break. Do not add DNA into the C-, C+ and background (applicable to FLASH PCR kits) tubes. Avoid paraffin layer break.

 Open the tube, add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips.

7.11 Add 5.0 µL of C- which has passed DNA isolation stage into C- and background (applicable to FLASH PCR kits) tubes. Add C+ into corresponding tube. Avoid paraffin layer break.

7.12 Spin tubes briefly (1-3 sec) at room temperature (not applicable to kits approved for use with Rotor-Gene Q thermal cycler).

7.13 Set the tubes to the Thermal Cycler.

Launch the Thermal Cycler software and run PCR according to instructions supplied with device, considering 35 µL reaction mix volume. See tables 3-7 to refer the cycling program and table 8 to refer the detection channels (applicable to Real-Time PCR kits). Using Tercyc cycler you need to choose «Fast active regulation» regulation algorithm.

Table 3. The PCR program for Tercyc Conventional PCR Thermal Cycler (applicable to **Conventional PCR kits and FLASH PCR kits)**

Step	For thermal cyclers with active regulation			Number of cycles
	Temperature, °C	Time		
		min	sec	
1	94	5	0	1
2	94 62	0 0	10 20	50
3	10	…	…	Storage

Note! When working with FLASH PCR detection Kits once prepared and amplified “BACKGROUND” tubes may be used many times at each PCR results detection with reaction tubes from the same lot. “BACKGROUND” tubes can be stored at temperatures between 2 °C and 8 °C and out of light for no longer than 1 month. During the detection procedure “BACKGROUND” tubes must be room temperature, for that take them out from refrigerator 1 hour before detection.

Table 4. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	94	5	00	1		Cycle
2	94	0	10	50		Cycle
	62	0	20		v	
5	10[1]	…	…	Holding		Holding
[1] – holding at 25°C is allowed						

Table 5. The PCR program for iCycler iQ5 thermal cyclers (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, °C	PCR/Melt Data Acquisition
1	1				
		1	05:00	94.0	
2	50				
		1	00:10	94.0	
		2	00:20	62.0	Real Time
3		…	…	10.0	Storage

Table 6. The PCR program for iCycler iQ5 thermal cyclers (with dynamic factor)

Cycle	Repeats	Step	Dwell time	Setpoint, °C	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	00:30	80.0	
		2	05:00	94.0	
2	5				
		1	00:20	94.0	
		2	00:30	62.0	
3	2				
		1	00:20	80.0	Real Time
PCR program					
4	45				
		1	00:10	94.0	
		2	00:20	62.0	Real Time
5		…	…	10.0	Storage

Table 7. The PCR program for Rotor-Gene Q Thermal Cyclers

Cycling	Temperature	Hold Time	Cycle Repeats
Cycling	80 °C	300 sec	1 time
Cycling 2	94 °C 58 °C 62 °C[3]	10 sec 5 sec 25 sec	50 times

Table 8. Detection channels

	Specific product	IC
DTprime, DTlite and IQ5	FAM	HEX
Rotor-Gene Q	Green	Yellow

8. CONTROLS

Table 9.

Control	The controlled step	Result		Interpretation
		Specific signal is present	Specific signal is absent	
C+	PCR	+	+	Valid
		-	-	Invalid
C-	PCR and DNA extraction	+	+	Invalid
		-	-	Valid
IC	PCR and DNA extraction	+	+	Valid
		-		Valid
			-	Invalid

[3] -take the measurement.

The sample is considered positive if the signal for specific DNA is present. The signal for IC could be absent in samples with high concentration of specific DNA due to competitive priming.

The sample is considered negative if the signal for specific DNA is absent and for IC is present.

If the signal for C- is present, whole tests of current batch considered false. Decontamination required.

9. DATA ANALYSIS

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers or Fluorescence Readers the analysis performed automatically. In all other cases the analysis is based on the presence or absence of specific signal. The controls should be also considered to exclude false positive and false negative results (see p. 8 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

The analysis performed automatically.

The interpretation should be performed in accordance with table 10, 11.

Table 10. Results with **HBV Conventional PCR detection Kit**

Specific product (295 bp)	Internal control (560 bp)	Interpretation
Samples		
+	Not considered	Positive
-	+	Negative
-	-	uncertain
C+		
+	Not considered	Positive
C-		
-	+	Negative

Table 11. Results with **HBV Flash** and **Real-time PCR detection Kits**

HBV FLASH PCR detection Kit	**Test samples**		**Interpretation**
	HBV Real-time PCR detection Kit		
	Fam/Green	**Hex/Yellow[4]**	
"+"	Cp (Ct) is specified	Not considered	Positive
"-"	Cp not specified (for iQ N/A)	Cp (Ct) 29-34/Ct <36	Negative
"uncertain"	Cp not specified (for iQ N/A)	Cp not specified (for iQ5 N/A)	uncertain
C+			
"+"	Cp (Ct) <34	Not considered	Positive
C-			
"-"	Cp not specified (for iQ N/A)	Cp (Ct) 29-34/Ct <36	Negative

[4] - if Cp (Ct) HEX more than specified the result is invalid!

10. TROUBLESHOOTING

Table 12.

	Specific signal +	Specific signal -	Possible cause	Solution
C+	-	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	+	Contamination	Dispose current batch Perform decontamination procedures
IC		-	PCR inhibition	Repeat whole test

If you face to any undescribed issues contact our representative.

11. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 month from the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

All components of the **HBV PCR detection Kit** (except PCR-mix and C+) must be stored at temperature from minus 18 °C to minus 22 °C over the storage period. The PCR-buffer and mineral oil can be stored at temperatures between 2 °C and 8 °C.

The PCR-mix, C+ and *PREP-NA* DNA/RNA Extraction Kit must be stored at temperatures between 2 °C and 8 °C over the storage period.

Transportation can be held by all types of roofed transport with adherence to above mentioned temperature requirements.

An expired **HBV PCR detection Kit** must not be used.

We strongly recommend following the instructions to get robust and reliable results.

The conformity of the **HBV PCR detection Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our customer service by quality issues of the **HBV PCR detection Kit**:

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12. SPECIFICATIONS

a. **Analytical specificity:** the **HBV PCR detection Kit** allows detection of all known HBV subtypes. The samples containing HBV will be defined as positive. The samples not containing HBV will be defined as negative.

b. **Sensitivity:** not less than 200 copies of HBV DNA per 1 mL of blood plasma.

c. **Diagnostic sensivity:** 99,8**%**.

d. **Diagnostic specificity:** 100**%.**

The claimed specifications are guaranteed when DNA extraction is performed with *PREP-NA* DNA/RNA Extraction Kit.

13. QUALITY CONTROL

"DNA-Technology, Research&Production" LLC declares that. the quality control procedures performed in accordance with ISO ISO 9001:2008 and ISO 13485:2003

14. KEY TO SYMBOLS


Caution


Manufacturer


Consult instructions for use

Negative control


Date of manufacture

Positive control


Expiration date

Cataloque number

In vitro diagnostic medical device

Sufficient for

Batch code


Temperature limitation

Version


Upper limit of temperature

Annex A

Nomogram and formula for calculation of relative centrifugal force (RCF) in the speed of rotation (RPM) depending of the rotor diameter