ANCHOR

EBV PCR Kit

Instructions for Use

Anchor EBV PCR Kit

CE

Quantitative or Qualitative

Real Time PCR Kit

for *in vitro* diagnostic use

IVD For *in vitro* diagnostic use

REF A1400

Σ 100

HB A1410-UK – 25.05.2022

QG A1411-UK – 25.05.2022

-30°C to -15°C

ANCHOR Diagnostics GmbH
Grandweg 64
D-22529 Hamburg

compatible with

LightCycler 480 II (Roche)

cobas z 480 Analyzer (Roche)

CFX96 (Bio-Rad)

Rotor-Gene Q (QIAGEN)

QuantStudio 5 (Applied Biosystems)

Mic qPCR (Biomolecular Systems)

1 Contents

2 Intended Use

The Anchor EBV PCR Kit is an in vitro nucleic acid amplification test based on Real Time PCR technology for the in vitro nucleic amplification test for the quantitative or qualitative detection of EBV (Epstein-Barr-Virus, Human gammaherpesvirus 4) DNA, isolated from human EDTA-Plasma or cerebrospinal fluid.
The product is intended to be used by professional operators, such as technicians and physicians who are trained in molecular biological techniques.

3 Product Description

The Kit constitutes a ready-to-use system for the amplification, detection and quantitation of EBV-specific nucleic acids.
In addition, a heterologous amplification system (Internal Control) is included to supervise the success of the sample extraction procedure and to identify possible inhibition of the amplification reaction.
Probes linked to distinguishable fluorescent dyes enable the parallel detection of EBV specific nucleic acids and the Internal Control in two corresponding detector channels of the Real Time PCR instrument.
The Quantitation Standards QS1-4 EBV contain defined concentrations of artificial DNA bearing the EBV target sequence. They are calibrated against the 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (NIBSC code 09/260) and can be used individually or as a whole set together with the Negative Control DNA 2 to monitor the integrity of the analyte-specific reagents of the kit and the proper performance of the reaction. When the Quantitation Standards QS1-4 EBV are used as a whole set, they allow to quantitate the EBV DNA present in a test sample.

4 Kit Components

Master A and Master B reagents contain all necessary components to allow PCR mediated amplification and target detection of EBV specific DNA and Internal Control in one reaction setup.
The Quantitation Standards QS1-4 EBV and NC (Negative Control) DNA 2 are supplied with the IC (Internal Control) DNA 2 already incorporated (see also section 9.2.1 Master Mix Set-Up).
The reagents provided with the kit allow the preparation of 100 reactions.

Master A EBV	Master B EBV	IC DNA 2	! QS1-4 EBV	! NC DNA 2
A1401	A1402	A0022	A1403-1/2/3/4	A0032
4 Vials	4 Vials	1 Vial	1 Vial each	1 Vial
4x 125 µL	4x 125µL	1000 µL	4x 200 µL	200 µL
Contains: Buffer, Bovine Serum Albumin, Polymerase	Contains: Buffer, Salt, Nucleotides, Target- and IC-specific Oligonucleotides	Contains: Buffer, IC-specific synthetic Polynucleotide	Contains: Buffer substance, Target-specific synthetic Polynucleotide	Contains: Buffer substance, IC-specific synthetic Polynucleotide

! INTERNAL CONTROL INSIDE !

5 Storage and Stability

- The Anchor EBV PCR Kit is shipped on dry ice and should be stored at –30 to –15°C upon receipt.
- The components are stable until the expiration date stated on the label.
- Do not use components of the kit that have passed their expiration date.
- Store EBV DNA-positive and/or potentially positive material separated from the kit.
- Repeated thawing and freezing of the Master reagents of > 3x should be avoided, as this may reduce the assay performance. For the Quantitation Standards QS1-4 EBV, the NC DNA 2 and the IC DNA 2, thawing and freezing cycles up to 4x are allowed. Alternatively, storage between +2 to +8°C for up to 14 days is possible.
- Due to the components used it might be possible that Master vials do not always freeze completely after initial thawing. This is not a matter of concern and does not influence the stability or performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Label aliquots clear and unambiguously to avoid a mix-up of reagents.
- During PCR set up the reagents should be kept cooled at +2 to +8°C – use cooling block.
- Do not store Master A and Master B EBV more than 3 h at +2 to +8°C.
- Protect all reagents from extensive light exposure.

6 Material Required but Not Provided

- Nucleic acid purification system
- Real Time PCR instrument
- Appropriate PCR reaction vessels and related accessories
- Cooling block (for reaction setup)
- Benchtop centrifuge (rotor holding 2 mL reaction tubes)
- Vortex mixer
- Pipettes (variable volume)
- Single-use pipette filter tips
- 1.5 mL or 2 mL reaction tubes (for Master mix set-up)
- Single-use gloves (powder-free)

Use all materials and equipment according to the manufacturer's instructions. Maintain and calibrate the equipment as recommended.

7 Limitations

- Strict compliance with the user manual is required for optimal PCR results.
- Any diagnostic results generated must be interpreted in conjunction with other clinical and/or laboratory findings.
- The presence of PCR inhibitors may cause invalid results.
- Occurrence of mutations within the target region might result into a reduced sensitivity, false quantitation or a complete detection failure.
- Following good laboratory practices is crucial for the successful usage of the product.
- Appropriate handling of the reagents is essential to avoid contaminations or impurities.

8 Warnings and Precautions

- For *in vitro* diagnostic use.
- Use of this product is limited to personnel specially instructed and trained in the techniques of Real Time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as potentially infectious and/or biohazardous material in accordance with safe laboratory procedures.
- The Anchor Master A EBV contains a bovine sourced potentially infectious component (albumin). The bovine plasma is sourced from New Zealand or USA, which are recognized by the world organization for animal health Office International des Epizooties (OIE, Paris) as having a negligible BSE risk.
- Wear protective single-use gloves, a laboratory coat and eye protection when handling specimens or kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free single-use pipette tips with aerosol barriers.
- Use separated working areas for (1) specimen preparation, (2) PCR reaction set-up and (3) amplification/detection activities.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Discard sample and assay waste according to your local safety regulations.

9 Workflow

9.1 Sample Preparation

9.1.1 Sample Matrix

The recommended patient sample matrix for sample preparation input is:

- human EDTA-plasma
- cerebrospinal fluid

The blood draw should be done using commercially available standard blood collection systems for EDTA-plasma (e.g. Sarstedt, Becton Dickinson, Greiner or equivalent). Tube contents should be mixed directly after sample collection according to manufacturer's instructions. For separation of EDTA-plasma, whole blood should be centrifuged according to the instructions provided by the manufacturer of the collection system within 24 hours after collection.

If a sample is not directly processed, store samples according to manufacturer's instructions (Collection Tube, Transport Tube, Sample Preparation Kit) before use. For shipment, follow the local and national regulations for the transport of biological material.

Storage recommendations:
Although several studies reported a relative stability of herpes viruses and their nucleic acids (Hasan et al. & Smit et al.) - if not specified by related manufacturer's instructions - storage of clinical samples under refrigerated conditions (+2 °C to +8 °C) should not exceed more than 3-4 days.
For long term storage clinical materials and purified nucleic acids should be frozen (at least -30 °C to -15 °C).

Short-Term Stability of Pathogen-Specific Nucleic Acid Targets in Clinical Samples
Mohammad R. Hasan, Rusung Tan, Ghada N. Al-Rawahi, Eva Thomas, Peter Tilley
Journal of Clinical Microbiology Nov 2012, 50 (12) 4147-4150

Comparison of collection methods for molecular detection of a-herpes viruses and Treponema pallidum, including evaluation of critical transportation conditions.
Pieter W. Smit, Titia Heijman, Meriem el Abdallaoui, Sylvia M. Bruisten Heliyon 5 (2019) e01522

9.1.2 Sample Preparation

Purified DNA is the sample input material for the Anchor EBV PCR Kit. It has to be ensured that the chosen nucleic acid purification method is compatible with Real-Time PCR technology. The extraction has to be executed according to the manufacturer's instructions.

The diagnostic applicability of the Anchor EBV PCR Kit has been shown using the following sample preparation systems:

Sample Preparation systems
NucliSENS® easyMag® System (bioMérieux)
EMAG® (bioMérieux)
EZ1 Advanced XL / EZ2 Connect (QIAGEN)
QIAcube Connect (QIAGEN)
QIAsymphony® SP (QIAGEN)
MagNA Pure 96 System (Roche)
MagNA Pure Compact (Roche)
Maxwell® 16 / RSC Instruments (Promega)
KingFisher Systems (Thermo Fisher Scientific)
SEEPREP32™ (Seegene)
GenoXtract® (Hain Lifescience)

ⓘ If sample eluates are not directly used for PCR analysis, store eluates at -30 to -15 °C. In case of using eluates repeatedly, avoid frequent thaw/freeze cycles (no more than 3 cycles).

ⓘ Eluates should be labelled clearly and unambiguously to avoid a mix-up of samples.

9.1.3 Internal Control

The Internal Control DNA 2 provided with the Anchor EBV PCR Kit should be co-purified with the nucleic acid of interest to monitor sample preparation efficiency and quality.

ⓘ The Internal Control DNA 2 MUST NOT be added directly to the clinical sample.

Always add the Internal Control DNA 2 after lysis buffer has been added to the sample.

The required volume of Internal Control DNA 2 per sample purification is defined by the chosen elution buffer volume.

Ten percent of the elution buffer volume used should be added to the sample/lysis mixture.

Examples:

- Elution buffer per sample: 200 µL -> IC DNA 2 volume: 20 µL
- Elution buffer per sample: 60 µL -> IC DNA 2 volume: 6 µL

ⓘ Secure the elimination of residual ethanol before elution of nucleic acids. Ethanol may inhibit the amplification process.

If no co-purification of the Internal Control is planned and the IC DNA 2 should be used only as an inhibition control of the reaction, either the amount of IC related to the used elution volume could be added to each eluate or 1.5 µL of the IC DNA 2 / per reaction should be added to the master mix (see section 9.2.1 Master Mix Set-Up).

9.2 PCR Preparation

9.2.1 Master Mix Set-Up

ⓘ Consider configuring the run settings of the PCR cycler software to have the instrument ready before starting the PCR reaction preparation (Refer to section 9.3 PCR Cycler Configuration).

Prepare the Master Mix step by step:

- Thoroughly thaw Master components A and B.
- Mix Master A and B by gentle pipetting or short pulse-vortexing.
- Spin Master A and B shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- According to your preferred workflow follow one of the pipette schemes below to mix Master A and B using a 1.5 mL or 2 mL reaction tube:

IC DNA 2 present in sample eluates – NO IC DNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A EBV	5 µL (X)	55 µL	Y µL
Master B EBV	5 µL (X)	55 µL	Y µL
Volume Master Mix	10 µL	110 µL	Z µL

*10 reactions + 10%
** See formula on next page

IC DNA 2 to be used as inhibition control only – IC DNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A EBV	5 µL (X)	55 µL	Y µL
Master B EBV	5 µL (X)	55 µL	Y µL
IC DNA 2	1.5 µL (X)	16.5 µL	Y µL
Volume Master Mix	11.5 µL	126.5 µL	Z µL

*10 reactions + 10%
** See formula on next page

We recommend calculating for an additional volume of at least 10% to compensate potential loss during pipetting. The needed volume will be calculated by using the following formula:

** $N \times X\mu L x 1{,}1 = Y$

N = Number of reactions
X = Volume of component per reaction
Y = Total volume of component
Z = Total volume of Master Mix

- Mix prepared Master Mix by gentle and short pulse-vortexing.
- Spin Master Mix shortly with a benchtop centrifuge to remove residual droplets from tube lids.

It is recommended to test all 4 Quantitation Standards and the Negative Control at least once in each PCR run for quantitative purposes. For qualitative analyses, the use of QS3 EBV as Positive Control is recommended. For further information, see also chapters 9.4.1 and 9.4.2, respectively.

Quantitation Standards QS1-4 EBV and the Negative Control DNA 2 already contain the IC DNA 2 in a ready-to-use concentration. No addition of IC necessary!

If you want to use a Master Mix preparation with added IC DNA 2 (as inhibition control) in combination with the QS1-4 and NC DNA 2, be aware that the IC signal of the controls will slightly shift towards a lower CT value in comparison to the IC signal of the controls using a Master mix without additional IC.

9.2.2 PCR Reaction Set-Up

ⓘ Always use a cooling block for the preparation of the PCR reaction mix.

Prepare the Reaction Mix step by step:

- If previously stored frozen, thaw eluates containing nucleic acid (and IC DNA 2) thoroughly.
- Mix eluates by gentle pipetting or brief pulse-vortexing.
- Spin eluates shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- Pipette **10 µL of Master Mix** (see section 9.2.1 Master Mix Set-Up) into suitable reaction vessels for PCR analysis. This is also valid for Master Mix spiked with IC DNA 2.
- Add **15 µL of eluate** or control (Quantitation Standards QS1-4 EBV or Negative Control DNA 2). **Mix well by repeated up and down pipetting.**
- Close reaction vessels securely with the appropriate sealing system.
- Immediately transfer closed and ready-to-use reaction vessels to the Real Time PCR instrument. Avoid any delays!

ⓘ **Carefully handle reaction vessels during transfer to avoid mix up of samples.**

ⓘ **Complete mixing of Master Mix reagents with a sample or control during reaction set up should be unconditionally secured by repeated up and down pipetting!**
This is essential to achieve an optimum amplification curve performance!!!

Master Mix	+	Eluate / Control	=	Reaction Mix
10 µL		15 µL		25 µL

9.3 PCR Cycler Configuration

The Anchor EBV PCR Kit has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 (Applied Biosystems)	≈ 28 min.
LightCycler 480 II (Roche)	≈ 30 min.
Cobas z 480 Analyzer (Roche)	≈ 30 min.
CFX96 (Bio-Rad)	≈ 33 min.
Rotor-Gene Q (QIAGEN)	≈ 39 min.
Mic qPCR (BMS)	≈ 33 min.

The listed run times for the different instruments are effectively measured durations and can differ from what is displayed on the graphical user interface of the individual instrument software. For basic information concerning set-up and programming of the respective Real Time PCR instrument, refer to the instrument-specific manual.

9.3.1 Temperature Profile

Temperature cycling profile for **QuantStudio 5, LightCycler 480 II, Cobas z 480 Analyzer, CFX96 and Rotor-Gene Q:**

Cycling	95°C	1 sec	x 40
	65°C *	2 sec	
	72°C	1 sec	

* Fluorescence acquisition for EBV and IC

Temperature cycling profile for **Mic qPCR:**

Cycling	95°C	1 sec	x 40
	63°C *	2 sec	
	72°C	1 sec	

* Fluorescence acquisition for EBV and IC

Reaction Volume: 25 µL

9.3.2 Specific PCR Cycler Settings

The following table contains PCR cycler-specific recommendations for the basic configuration of the run settings.
For additional information regarding the cycler settings recommended plastics, colour compensation, gain optimisation settings, etc. do not hesitate to contact us directly (see section 12 Technical Assistance & Contact Information).

Instrument	Target	Detection channel	Recommendations / Requirements
LightCycler® 480 II (Cobas z 480 Analyzer)	EBV	465/510	**Run Settings:** ▪ Block size: 96 ▪ If clear plates are used, the sensor of the LightCycler® has to be disabled by selecting the Clear Plates option in the software before the run is started. **Consumables:** ▪ LC480 Multiwell Plate 96, white (Roche Mat. No. 04729692001) ▪ LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001) ▪ LC480 Sealing Foil (Roche Mat. No. 04729757001)
	IC	533/580 (540/580)	
Bio-Rad CFX96	EBV	FAM	**Consumables:** ▪ Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655) ▪ Optical flat 8 Cap Strip for 0.2ml (Mat. No. TCS0803) ▪ 0.2 ml 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad Mat. No. TLS 0851) ▪ 8-strip optical clear flat caps (Sarstedt Mat. No.65.1998.400)
	IC	HEX	

Instrument	Target	Detection channel	Recommendations / Requirements
Rotor-Gene Q	EBV	Green	**Run Settings:** ▪ Use 72-Well Rotor ▪ Perform Auto-Gain optimisation before 1st acquisition. **Consumables:** ▪ Strip Tubes and Caps, 0.1 ml (QIAGEN Mat. No 981103)
	IC	Yellow	
QuantStudio™ 5	EBV	FAM	**Run Settings:** ▪ Block Type: 96-Well 0.1-mL Block ▪ Experiment Type: Standard Curve Chemistry: TaqMan® Reagents ▪ Run Mode: Fast ▪ Plate attributes: Passive Reference - None **Consumables:** ▪ 96-Well Fast Thermal Cycling Plates (Life Technologies Mat.No. 4346907) ▪ MicroAmp™ Optical Adhesive Film (Life Technologies Mat. No. 4311971) ▪ 96-Well-PCR-Plate, Skirted, „Low Profile“, white (Starlab Mat. No. E1403-5209) ▪ Xtra-Clear Advanced Polyolefin StarSeal (qPCR) (Starlab Mat. No. E2796-9795)
	IC	HEX	
Mic qPCR	EBV	Green	**Run Settings:** ▪ Temperature Control: Standard TAQ **Consumables:** ▪ Mic Tubes and Caps (Mat. No.68MIC-60653)
	IC	Yellow	

When preferring a quantitative analysis of the clinical samples, the Quantitation Standards QS1-4 EBV must be labelled as standards within the instrument software and assigned with their appropriate concentrations.

Quantitation Standard	Concentration [IU/µL]
QS1 EBV	50,000
QS2 EBV	5,000
QS3 EBV	500
QS4 EBV	50

9.4 Data Analysis

The following table contains cycler-specific references for the configuration of analysis settings. They could serve as an initial orientation. Depending on local cycler- and workflow-related differences adaptations might be necessary.
For additional information concerning data analysis, refer to the instrument-specific manual of the respective Real Time PCR instrument or contact us (see section 12 Technical Assistance & Contact Information).

Instrument	Recommendations
LightCycler® 480 II (Cobas z 480 Analyzer)	**Analysis Settings:** ▪ Abs Quant/Fit Points ▪ Color Comp (off) ▪ Mean ▪ High Confidence ▪ Threshold LightCycler 480 II:: - 465/510: 0.65 - 533/580: 2.50 ▪ Threshold Cobas z 480 Analyzer: - 465/510: 0.58 - 540/580: 1.45
Bio-Rad CFX96	**Analysis Settings (all channels):** ▪ Baseline Substracted Curve Fit ▪ C(t) Determination Mode: Single Threshold ▪ Baseline Threshold: - Baseline Cycles: Auto Calculated - Single Threshold - FAM: 1,000 - HEX: 350
Rotor-Gene Q	**Analysis Settings (all channels):** ▪ Quantitation ▪ Linear Scale ▪ Dynamic Tube ON ▪ Threshold: - Green: 0.5 - Yellow: 0.8
QuantStudio™ 5	**Analysis Settings (all channels):** ▪ Plot Type: ΔRn vs Cycle ▪ Graph Type: Linear ▪ Baseline Start/End: 3/15 ▪ Threshold: - FAM: 200,000 - HEX: 50,000
Mic qPCR	**Analysis Settings (all channels):** ▪ Graph Type: Linear ▪ Method: Dynamic ▪ Ignore Cycles Before: 3 ▪ Threshold Start: 1 ▪ Exclusion: None ▪ Threshold Level: - Green: 1.60 - Yellow: 1.30

9.4.1 Qualitative Analysis

For a valid run and as a prerequisite for the interpretation of the individual clinical sample results, the following requirements have to be met by the included kit controls:

Channel/Target	EBV	IC
QS3 EBV[1]	+	+
NC DNA 2	-	+

If one of the conditions has failed, result interpretation of clinical sample results might be flawed. In case of kit control failure, it is recommended to repeat the PCR run.

In case of a valid run, the following result interpretation can be made:

Qual. result	EBV	IC
EBV DNA positive	+	+/-
EBV DNA negative	-	+
Invalid	-	-

A positive result for EBV DNA does not necessarily require a positive signal for the IC since high concentrations of the respective target nucleic acid can result in a competitive inhibition of the IC amplification.

An invalid result for a clinical sample can be due to PCR inhibition or a failure during the nucleic acid isolation procedure. In such cases, it is recommended to dilute the nucleic acid extract 1:10 (recommended to be done in elution buffer, if possible) for a PCR retest or to repeat the nucleic acid isolation procedure. Note that the dilution of the nucleic acid extract might also lead to a reduction of the target nucleic acid concentration below the limit of detection of the Anchor EBV PCR Kit.

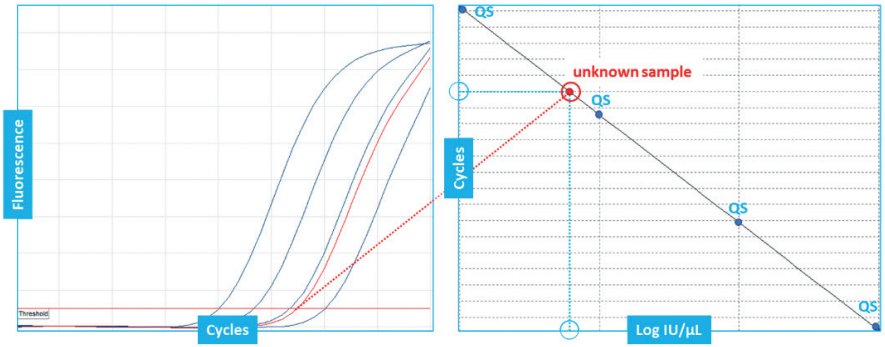
[1] It is recommended to use QS3 EBV as Positive Control

9.4.2 Quantitative Analysis

For a valid run and as a prerequisite for the interpretation of the individual clinical sample results, the following requirements have to be met by the included kit controls:

Channel/Target	EBV	IC	Correlation Coefficient r^2	Slope m
QS1-4 EBV	+	(+)[2]	≥0.99	-3.0 to -3.6
NC DNA 2	-	+	-	-

If one of the conditions has failed, the quantitative interpretation of the clinical sample results might be flawed. In such cases, it is recommended to repeat the PCR run.
If all criteria are met, the standard curve generated with QS1-4 EBV of known concentrations can be used to determine the EBV DNA load present in a clinical sample.



The concentration of any target DNA within a sample eluate will be quantified according to the formula

$$Conc. = 10^{\frac{C_t - b}{m}}$$

where *m* is the slope of the standard curve and *b* the y-intercept.

[2] QS1 and QS2 EBV are excluded from this rule. The presence of high concentrated artificial nucleic acids in this Standard can result in a competitive inhibition of the IC amplification.

The results are displayed in IU/ µL. To calculate the concentration of EBV DNA in the original clinical sample in IU/mL, the concentration factor of the applied sample preparation system must be considered:

$$Sample\ \frac{IU}{mL} = Eluate\ \frac{IU}{\mu L} \times \frac{Volume\ Eluate\ [\mu L]}{Volume\ Sample\ input\ [mL]}$$

For inter-assay result comparison, a conversion factor needs to be established to adjust quantitative results considering workflow-specific performance characteristics. If you plan to establish such a conversion factor please contact us for technical assistance, if required (see section 12 Technical Assistance & Contact Information).

10 Performance Data

10.1 Analytical Performance

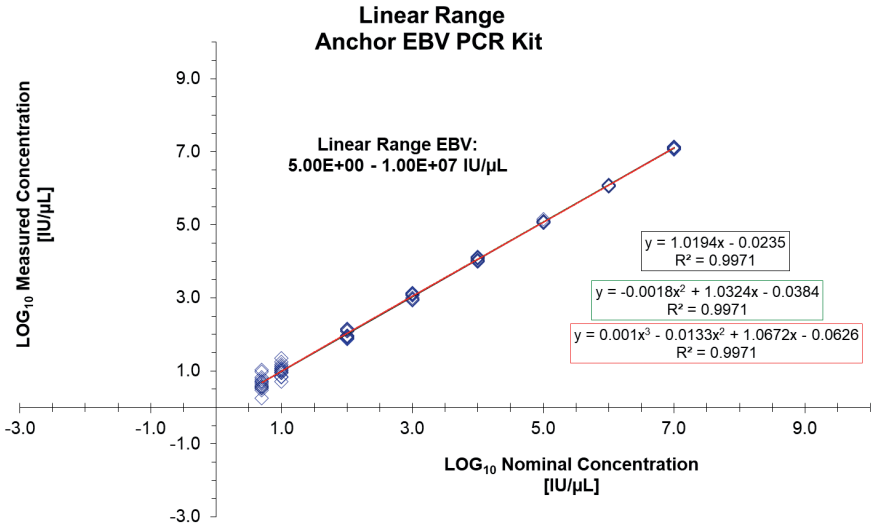
10.1.1 Sensitivity

The LOD for the Anchor EBV PCR Kit was determined by undertaking a probit analysis on the Rotor-Gene Q platform. A dilution series of different concentration levels for EBV strain B95-8 DNA (NIBSC 09/260) was used. Each dilution level was tested with overall 24 replicates using 3 different PCR reagent lots across 3 different days, executed by 2 different persons on 2 different instruments.
The LOD value determined on the Rotor-Gene was then confirmed or re-evaluated on the other 5 instruments.

Instrument	LOD	Unit
Rotor-Gene Q	1.27	IU/µL
QuantStudio 5	1.27	IU/µL
LightCycler 480 II	1.27	IU/µL
Cobas z 480 Analyzer	1.27	IU/µL
CFX96	1.27	IU/µL
Mic qPCR	1.27	IU/µL

10.1.2 Linearity

The linear range of the Anchor EBV PCR Kit was determined by testing a dilution series of artificial DNA comprising the EBV PCR target, ranging from 1.00E+07 IU/µL to 1.00E+00 IU/µL. The dilutions were analysed concentration-dependent with 4-8 replicates. All replicates of one dilution were tested in one PCR run. The linear range was determined with two Anchor EBV PCR Kit lots.
The picture below shows a scatter chart of measured concentrations plotted against their nominal concentrations. The correlation of data is described by linear, 2nd and 3rd order polynomial curve fitting.



The linear quantification range for the Anchor EBV PCR Kit was tested and confirmed to be from at least 5.00E+00 up to 1.00E+07 IU/µL. The Limit of Quantitation (LOQ) is therefore determined to be 5.00E+00 IU/µL.

10.1.3 Specificity

Triplicates of two different EBV culture strains were tested at a concentration near the 3x LOD of the Anchor EBV PCR Kit.

Strain	EBV
EBV strain B95-8	+
EBV strain P3HR1	+

Nucleic acid of selected pathogens with a concentration of ≈5.00E+03 copies/µL (alternative units CFU/µl or TCID50/µL) was added to the PCR reaction and tested in triplicates in the absence or presence of EBV DNA at its 3x LOD and 3x LOQ concentration on the Rotor-Gene Q.

Pathogen	- EBV	3x LOD EBV	3x LOQ EBV Δ Log_{10} IU/µL[3]
Adenovirus 2	-	+	-0.09
Adenovirus 4	-	+	-0.01
Adenovirus 5	-	+	-0.07
HSV-1	-	+	-0.10
HSV-2	-	+	-0.10
BK Virus	-	+	-0.06
JC Virus	-	+	-0.06
Cytomegalovirus	-	+	-0.04
Hepatitis A Virus	-	+	0.01
Hepatitis B Virus	-	+	-0.03
Human Herpesvirus 6A	-	+	-0.04
Human Herpesvirus 6B	-	+	-0.03
Human Herpesvirus 7	-	+	0.03
Human Herpesvirus 8	-	+	-0.03
Human Parvovirus B19	-	+	-0.17
Enterovirus 71	-	+	0.04
West-Nile Virus	-	+	0.08
VZV	-	+	0.00
Candida albicans	-	+	-0.16
Aspergillus fumigatus	-	+	-0.03
Aspergillus niger	-	+	0.01
Salmonella typhimurium	-	+	-0.06
Streptococcus pneumoniae	-	+	0.04
Cutibacterium acnes	-	+	-0.02
Staphylococcus aureus	-	+	0.01

[3] In relation to EBV DNA-only control

10.1.4 Precision

Precision testing was initially performed on the Rotor-Gene Q instrument. For intra-run variability, 3-6 replicates of each sample dilution were tested within one run using one instrument and reagent lot by one operator. For inter-run variability, 3-6 replicates of each sample dilution were tested within overall four runs using two instruments and one reagent lot by two operators across days. For inter-batch variability, 3-5 replicates of each sample dilution were tested within one run using one instrument and three reagent lots by one operator.

		QS1 EBV	
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	19.61	0.06	0.31
Inter-Run	19.63	0.05	0.26
Inter-Batch	19.57	0.08	0.39
Total	19.61	0.07	0.36
		QS4 EBV	
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	29.65	0.34	1.13
Inter-Run	29.43	0.27	0.91
Inter-Batch	29.47	0.26	0.89
Total	29.50	0.25	0.84
		QS EBV 3xLOQ (15 IU/µL)	
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	31.34	0.34	1.10
Inter-Run	31.49	0.24	0.77
Inter-Batch	31.52	0.27	0.86
Total	31.53	0.23	0.73

	WHO Int. Stand. EBV 3xLOQ (15 IU/µL)		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	30.44	0.45	1.49
Inter-Run	30.87	0.75	2.43
Inter-Batch	31.24	0.29	0.94
Total	31.02	0.68	2.18
	QS EBV 3xLOQ (15 IU/µL)		
Variability	AVE (IU/µL)	SD (IU/µL)	CV (%)
Intra-Run	13.00	3.46	26.65
Inter-Run	11.09	2.45	22.08
Inter-Batch	11.33	2.44	21.53
Total	10.91	2.16	19.76
	WHO Int. Stand. EBV 3xLOQ (15 IU/µL)		
Variability	AVE (IU/µL)	SD (IU/µL)	CV (%)
Intra-Run	21.83	5.56	25.49
Inter-Run	19.09	8.46	44.31
Inter-Batch	14.00	2.93	20.91
Total	17.15	7.74	45.14

Precision of the Anchor EBV PCR Kit in combination with the other instruments was evaluated for intra- and inter-run variability.

10.2. Clinical Performance

The clinical performance of the Anchor EBV PCR Kit for the qualitative and quantitative detection of EBV DNA in human EDTA-Plasma samples was evaluated comparatively at one study site against an EBV diagnostic workflow using a CE-marked PCR-Assay as reference standard.

245 banked clinical specimens were analysed with the Anchor EBV PCR Kit and with the comparator assays to determine their positive percent agreement (PPA) and negative percent agreement (NPA), respectively. Testing was done using the CFX96 PCR Cycler and the LightCycler 480 II instrument.

		Comparator	
	∑ 245	POS	NEG
Anchor	POS	37	4
EBV PCR Kit - CFX96	NEG	6	198

PPA: 86.1 % NPA: 98.0 %

		Comparator	
	∑ 245	POS	NEG
Anchor	POS	38	4
EBV PCR Kit - LC 480 II	NEG	4	199

PPA: 90.5 % NPA: 98.0 %

The clinical performance of the Anchor EBV PCR Kit for the qualitative and quantitative detection of EBV DNA in CSF samples was evaluated comparatively to one CE-marked diagnostic workflow involving overall 181 banked clinical CSF samples, 180 with valid test results.

These samples were analysed with the Anchor EBV PCR Kit and with the comparator assay to determine their positive percent agreement (PPA) and negative percent agreement (NPA), respectively.

Testing was done using the CFX96 PCR Cycler and the LightCycler 480 II instrument.

		Comparator	
	∑ 180	POS	NEG
Anchor	POS	62	5
EBV PCR Kit - CFX96	NEG	1	112

PPA: 98.4 % NPA: 95.7 %

		Comparator	
	∑ 180	POS	NEG
Anchor	POS	60	2
EBV PCR Kit - LC 480 II	NEG	3	115

PPA: 95.2 % NPA: 98.3 %

11 Quality Control

In accordance with the implemented ISO 13485-certified Quality Management System, each lot of the Anchor EBV PCR Kit is tested against predetermined specifications to ensure consistent product quality.

12 Technical Assistance & Contact Information

For any questions, a need for technical assistance or if you identify difficulties using our products do not hesitate to contact us:

phone: +49 40 520 14 830
email: support@anchor-diagnostics.com

13 Literature

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(19) ECIL-4: 2011 Update on the ECIL-3 guidelines for EBV management in patients with leukemia and other hematological disorders (https://www.leukemia-net.org/content/treat_research/supportive_care/standards_sop_and_recommendations/e4702/infoboxContent5845/ECIL4EBVupdatefinal.pdf)

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14 Symbols

IVD For *in vitro* diagnostic use

REF Product - Catalogue number


Contains sufficient reagents for <N> tests


Instructions for Use - Catalogue number and version


Consult Instructions for Use

QG Quick Guide - Catalogue number and version


Temperature limits for storage


Use by

LOT Batch code


Important Note


Manufacturer

Notes

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