

GeneProof Adenovirus PCR Kit



1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof Adenovirus PCR Kit	25 reactions	ADV/ISEX/025
GeneProof Adenovirus PCR Kit	100 reactions	ADV/ISEX/100



2. INTENDED PURPOSE AND USE

Indication	<i>In vitro</i> diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics, aid to diagnosis and monitoring test
What is Detected / Target	Adenovirus
Automated / Manual Detection	Manual
Type of Analysis	Qualitative and quantitative
Validated Specimen	Aspirate, plasma, stool*, swab, urine, whole blood (EDTA)
Testing Population	EU population
Intended User	For professional use in laboratories with trained staff
Test Principle	Real-time polymerase chain reaction (PCR) – amplification of the specific Target Sequence and detection using TaqMan probes with fluorophore-based detection

*NOTE: Only in combination with QIAamp DNA stool Mini Kit.

3. TECHNICAL SPECIFICATION

Target Sequence	E2B gene		
Analytical Specificity	Adenovirus, 100 %		
Analytical Sensitivity (LoD with 95% probability)	Sample processing	Sensitivity	Performed on
	GeneProof PathogenFree DNA Isolation Kit	81.41 cp/ml 2371.645 cp/ml	AcroMetrix™ Adenovirus Plasma Panel Qnostics ADV Molecular 'Q' Panel
Diagnostic Specificity	87.98 % (CI _{95%} : 82.16 % - 92.15 %)		
Diagnostic Sensitivity	100.00 % (CI _{95%} : 91.11 % - 100.00 %)		
Linear Range	10 ¹⁰ – 10 ^{2.5} cp/ml with precision of ± 0.5 log using AcroMetrix™ Adenovirus Plasma Panel 10 ¹⁰ – 5x10 ³ cp/ml precision of ± 0.5 log using Qnostics ADV Molecular 'Q' Panel		
Dynamic Range	10 ¹⁰ – LoD cp/ml (LoD varying according to the extraction and material used)		
Reporting Units	cp/ml		
Extraction/Inhibition Control	PCR inhibition and DNA extraction efficiency control by Internal Standard (IS)		
Metrological Traceability	Qnostics ADV Molecular 'Q' Panel		
Validated Extraction Methods	croBEE 201A Nucleic Acid Extraction Kit GeneProof PathogenFree DNA Isolation Kit QIAamp DNA stool Mini Kit		
Applied Instruments	Instrument Name	ADV	Internal Standard (IS)
	croBEE Real-Time PCR System	FAM	HEX
	AMPLiLab Real-Time PCR System	FAM	HEX
	Applied Biosystems 7300 / 7500 Real-Time PCR System	FAM	JOE
	AriaMx Real-Time PCR System	FAM	HEX
	BioQuant-96 Real-Time PCR System	FAM	HEX
	CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System	FAM	HEX
	Gentier 96E/96R Real-Time PCR System	FAM	HEX
	LightCycler® 480	FAM	HEX
	LineGene 9600 / 9600 Plus	FAM	HEX
	Mic qPCR Cyclor	FAM	HEX
	QuantStudio™ 3 / 5 Real-Time PCR System	FAM	VIC
	Rotor-Gene 3000 / Q	FAM	JOE
SLAN® Real-Time PCR System	FAM	HEX	
StepOne™ / StepOne Plus™ Real-Time PCR System	FAM	JOE	
Detection Channels	FAM (ADV), HEX/JOE/VIC (IS)		
External Quality Assessment	Regularly tested in INSTAND e.V. External Quality Assessment Panels - results at www.geneproof.com		

4. INTERFERENCES

The evaluation and settings of pathological values for interference testing was performed according to CLSI guidelines EP7-A2 and guidelines and recommendations of Czech Society of Clinical Biochemistry.

Endogenous and Exogenous Interferences

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
PLASMA					
Albumin	60 g/l	None	Haemoglobin	2 g/l	None
Bilirubin	342 µmol/l	None	Urea	42.9 mmol/l	None
Glucose	55 mmol/l	None	Uric acid	1.4 mmol/l	None
Caffeine	308 µmol/l	None	Prednisone	0.84 µmol/l	None
Ibuprofen	2425 µmol/l	None	Vancomycin	69 µmol/l	None
Fluconazole	245 µmol/l	None	Citrate	19 g/l	None
ASPIRATE					
Whole blood	2 % (w/v)	None	Mucin	60 µg/ml	None
URINE					
Bilirubin	1 %	None	Albumin	5 %	None
Urea	600 mmol/l	None	pH	pH 4	None
Glucose	1 %	None	pH	pH 9	None
Uric Acid	1.4 mmol/l	None	-	-	-
WHOLE BLOOD					
EDTA	2.2 g/l	None	Heparin	30 IU/ml	None
SWAB					
Whole blood	2 % (w/v)	None	Mucin	60 µg/ml	None
Sodium chloride	6.5 mg/ml	None	Phenylephrine	0.375 mg/ml	None
Oxymetazoline	0.0625 mg/ml	None	Budesonide	100 µg	Partial
Xylometazoline	0.25 mg/ml	None	Neomycin + Bacitracin	330 I.U. neomycin 25 I.U. bacitracin	None

NOTE: In the case of partial interference, inhibition may occur with the risk of a false negative result at a given concentration of interferent.

5. KIT CONTENT

Reagent	Content	Vial Title	Cap Colour	Guaranteed Volume [µl]	Number of Vials	
					ADV/ISEX/025 – for 25 rxn	ADV/ISEX/100 – for 100 rxn
Master Mix	Mixture of PCR enzymes, target specific primers and TaqMan probes in buffer	Master Mix ADV	Blue	750	1	4
Calibrator	DNA oligonucleotide in buffer	Calibrator Adenovirus 10 ⁵ cp/µl	Black	200	1	1
		Calibrator Adenovirus 10 ⁴ cp/µl	Brown	200	1	1
		Calibrator Adenovirus 10 ³ cp/µl	White	200	1	1
		Calibrator Adenovirus 10 ² cp/µl	Transparent	200	1	1
Internal standard	DNA oligonucleotide in buffer	Internal Standard Adenovirus	Red	1000	1	2

6. CALIBRATOR INFORMATION

The use of all 4 calibrators is necessary for correct sample quantification. The automatic quantification based on the analysis of calibrators is generated automatically as a part of analytical process performed in the PCR instrument. Each calibrator consists of target specific DNA. Each calibrator must be designated as „standard“ in the instrument (thermocycler). The value of each calibrator must be entered when samples are defined in the PCR plate set up in the data analysis software.

NOTE: In the case of qualitative detection, the Calibrator 10³ cp/µl serves as a positive control.

7. TRANSPORT AND STORAGE

Storage Conditions	(-20 ± 5) °C
Transport Conditions	-20 °C and below
In-use Stability	thaw a maximum of 5 times or use within 30 days after the first use of a particular vial, whichever comes first

8. ASSAY PROCEDURE

SPECIMEN COLLECTION, TRANSPORTATION AND HANDLING

1. Samples for DNA extraction must be collected and transported following professional guidelines, at temperature (5 ± 3) °C.
2. Samples for DNA extraction must be transported and treated by the laboratory in the shortest possible time (preferably within 24 hours).

NUCLEIC ACID PURIFICATION

3. Prepare specimens for the assay according to the corresponding extraction kit manual.
4. Thaw required amount of Internal Standard (IS or UNIC*) vials, mix and briefly centrifuge.
5. Add Internal Standard (IS or UNIC) directly into the sample at the beginning of the extraction process so that 1 µl of the resulting elution volume contains 0.1 µl of the IS:

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Standard (IS or UNIC)	2.5 µl	5 µl	10 µl	20 µl

6. Continue extraction according to the appropriate protocol.

NOTE:

In case of using *UNIC = GeneProof Universal Internal Control (more information in chapter 12. Additional Products), see Instruction for Use of GeneProof Universal Internal Control.

PCR SETUP PROTOCOL

7. Thaw required vials and reagents completely.
8. Gently vortex and briefly centrifuge all vials before setting up the PCR run.
NOTE:
Keep the reagents at (5 ± 3) °C for the shortest time possible until the PCR reaction is set up.
9. Add 30 µl of Master Mix into PCR tubes.
10. Add 10 µl of the extracted nucleic acid sample or 10 µl of Calibrator into the individual PCR tubes and mix by pipetting. The total reaction mix volume is 40 µl.
11. Close the tubes, centrifuge shortly, insert them into the real-time PCR device and amplify according to the following PCR profile.
NOTE:
It is recommended to perform at least 1 negative control and at least 1 full range of calibrators (for a quantitative kit) for each individual PCR run. Use your own negative control (not provided) in the form of nuclease-free water. For more information see chapter 10. Run Validity.

AMPLIFICATION PROFILE

Follow the thermocycler manufacturer's manual for setting the instrument and for analysis.

Universal PCR Profile

NOTE: The Universal PCR Profile is designed for parallel detection with other GeneProof PCR Kits.

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	Reverse Transcription/ UNG decontamination	42	15 min	1 cycle	
2	Initial denaturation	95	10 min	1 cycle	
3	Denaturation	95	5 s	45 cycles	FAM, HEX/JOE/VIC
	Annealing	60	40 s		
	Extension	72	20 s		

DNA PCR Profile

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination	37	2 min	1 cycle	
2	Initial denaturation	95	10 min	1 cycle	
3	Denaturation	95	5 s	45 cycles	FAM, HEX/JOE/VIC
	Annealing	60	40 s		
	Extension	72	20 s		

9. INTERPRETATION OF RESULTS

Channel FAM (ADV)	Channel HEX/JOE/VIC (IS)	Result	Interpretation
+	+	Valid	ADV positive
+	-	Valid	ADV positive
-	+ (C _t < 38)	Valid	ADV negative
-	+ (C _t > 38)	Invalid	-
-	-	Invalid	-

NOTE: For interpretation of PCR run see chapter 10. Run Validity.

10. RUN VALIDITY

OVERALL VALIDITY OF DETECTION

	Signal	Channel	Run validity	Recommendation
Calibrator 10 ³ (qualitative detection) or Calibrator Set (quantitative detection)	+	FAM	Valid	-
Calibrator 10 ³ (qualitative detection) or Calibrator Set (quantitative detection)	-	FAM	Invalid	Repeat PCR run
Negative control	-	FAM	Valid	-
Negative control	+	FAM	Invalid	Repeat PCR run

NOTE: If the issue persists, please contact Customer Support, see Contact information.

11. QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the viral load concentration in cp/ml for manual extraction (using GeneProof PathogenFree DNA Isolation Kit):

VLC – Viral load concentration [cp/ml]

SC - Sample concentration [cp/μl]

EV - Elution volume [μl]

IV - Isolation volume [μl]

$$VLC = \frac{SC \times EV \times 10^3}{IV}$$

To easily calculate pathogen concentrations using manual or automated extraction, you can use the calculator at www.geneproof.com

VALIDITY OF QUANTITATIVE DETECTION

Channel	Calibrators				Result	Recommendation
	10 ⁵	10 ⁴	10 ³	10 ²		
Target specific channel (FAM)	++++	+++	++	+	Valid exact quantification	-
Internal Standard channel (HEX/JOE/MIC)	+/-	+/-	+/-	+/-		
R ²	≥0.98					
Target specific channel (FAM)	++++	+++	++	+	Reduced quantification accuracy	Repeat PCR run
Internal Standard channel (HEX/JOE/MIC)	+/-	+/-	+/-	+/-		
R ²	<0.98					
Target specific channel (FAM)	No signal of one or more calibrators				Invalid quantification	Repeat PCR run
Internal Standard channel (HEX/JOE/MIC)	+/-	+/-	+/-	+/-		
R ²	N/A					

R² – Determination coefficient – parameter evaluating the quality of standard curve

NOTE: If the issue persists, please contact Customer Support.

12. ADDITIONAL PRODUCTS

GeneProof Universal Internal Control

GeneProof Universal Internal Control (UNIC) is intended to be used as the Internal Control/ Standard for the microbiological GeneProof PCR kits and as an alternative product to Internal Controls/ Standards included in the GeneProof microbiological PCR kits. The UNIC works only in combination with GeneProof PCR kits. It is intended to simplify the user's workflow in cases where multiple detection kits with single extract are used. For more details see the Instruction for Use for UNIC.

Product	REF
GeneProof Universal Internal Control	UNIC/GP/050

NOTE: IS is applied to the solution only once. Add UNIC instead of IS from the package of the PCR kit. Do not add IS and UNIC to the same sample at the same time.

13. MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

CONSUMABLE MATERIAL

96-well reaction plates or PCR tubes (0.2 ml volume), pipette tips with filters, powder-free gloves, biohazard waste bin, nuclease-free water

DEVICES

Real-time PCR instrument (see chapter 3. Technical Specification), nucleic acid extraction system or kit (see chapter 3. Technical Specification), desktop centrifuge (for 0.2 ml PCR tubes or 96-well plates), vortex mixer, freezer (-20 ± 5) °C, refrigerator (5 ± 3) °C, cooling rack

14. WARNINGS, PRECAUTIONS AND PROCEDURE LIMITATIONS

- Patient management decisions should never be made based solely on the results from this test. Other laboratory and clinical factors must also be considered in making clinical decisions.
- Any serious incident occurred in relation to the using of GeneProof PCR Kit shall be reported to the manufacturer and to the competent local authority.
- Use all necessary protective equipment (protective disposable gloves, a laboratory coat and eye protection) when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of the reagents when removing aliquots from reagent vials.
- Use RNase- and DNase-free filter pipette tips only.
- Use new tips for each pipetting step.

- Use separate working places for sample preparation / nucleic acid extraction and amplification reactions. Never introduce an amplified product in reagent and/or nucleic acid extraction (sample preparation) area.
- Close the kit components vials immediately after use and never interchange lids.
- Do not pool reagents from different lots or from different vials within the same lot.
- Do not substitute the reagents between different lots.
- Do not use reagents from damaged or leaking vials.
- Dispose of unused reagents and waste in accordance with national, federal, state or local regulations.
- Be very careful when handling the Positive Control or the clinical material; incorrect handling could result in contamination and the consequent impairment of the kit components! The manufacturer is not responsible for the kit impairment due to incorrect handling.











Procedure Limitations:

- Read the whole Instruction for Use properly before starting the manipulation. Not following these instructions can lead to an erroneous result which can cause misdiagnosis or inappropriate treatment!
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- Do not use kit after the expiry date.
- The presence of UNG decontamination step reduces the risk of lower levels of amplicon contamination. However, contamination from very high levels of amplicons can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Instruction for Use. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This product is designed for use with the applied PCR instruments and validated extraction methods mentioned in chapter 3. Technical Specification.

Clinical Limitations:

- Detection of ADV DNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors.
- Use only with validated specimens (see chapter 2. Intended Purpose and Use) otherwise incorrect results could occur.

15. EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
	This product complies with the relevant EU requirements		Lot number
	<i>in vitro</i> diagnostic medical device		Contains sufficient amount for n-tests
	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
	Read Instruction for Use		Date of Manufacture (for selected territories only)

Customer Support

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