Package insert

GeneProof
Molecular diagnostics for your routine

GeneProof Adenovirus PCR Kit

CE IVD

In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid isolation and PCR inhibition control		
REF	**ADV/ISIN/025** 25 rxn	**ADV/ISIN/050** 50 rxn	**ADV/ISIN/100** 100 rxn	**ADV/ISEX/025** 25 rxn	**ADV/ISEX/050** 50 rxn	**ADV/ISEX/100** 100 rxn
MasterMix Adenovirus	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
CALIBRATOR Adenovirus 10^4 copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR Adenovirus 10^3 copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR Adenovirus 10^2 copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR Adenovirus 10^1 copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Internal Standard Adenovirus	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits should be transported and stored at temperatures between -85 °C and -10 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

TECHNICAL SPECIFICATION

Gene Target	*E2B* gene
Specificity	Adenovirus A-G
Sensitivity (LOD)	reaches 250 copy/ml with probability of 95 %
Accuracy of Measurement	within the range of 10^4 - 250 copy/ml the detection accuracy is 1 log
Linear Range of Measurement	10^{10} - 250 copy/ml
Sample Material	whole blood in EDTA, plasma, urine, aspirate, stool, nasopharyngeal and oropharyngeal swab
Reporting Units	copy/ml
Quality Control	periodically tested in External Quality Assessment - QCMD and INSTAND e.V.

METHOD PRINCIPLES

The Adenovirus detection is based on the amplification of highly conservative DNA sequence of *E2B* gene and measuring the amplification product concentration using PCR process and fluorescence labelled probes. Adenovirus presence is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction (ISIN version) and also the DNA extraction quality (ISEX version). IS positive amplification is detected in the fluorescence channel for the HEX fluorophore. The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR by amplification products. The kit performs very sensitive detection of all Adenovirus A-G basic groups and all sequentially known Adenovirus serotypes from clinical material (whole blood in EDTA, plasma, urine, aspirate, stool, nasopharyngeal, oropharyngeal swab). The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detections.

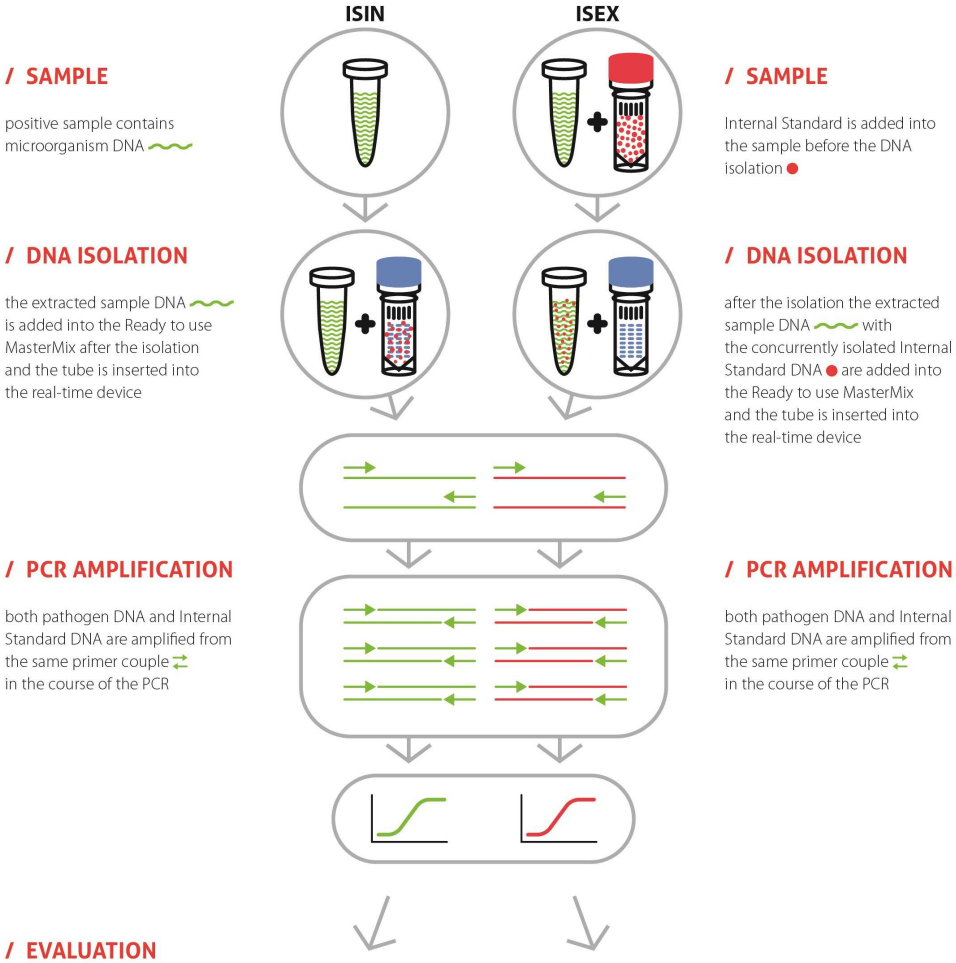
ISIN version

Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

ISEX version

Internal Standard is provided as independent item within the package. This PCR kit version enables both PCR inhibition control and nucleic acid purificaion process efficiency control.

MICROBIOLGICAL DNA DETECTION TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Sample types suitable for Adenovirus detection are whole blood in EDTA, plasma, urine, aspirate, stool, nasopharyngeal and oropharyngeal swabs. Transport samples in temperature range +2 °C to +8 °C and for long term storage keep samples in frozen temperature range -85 °C to -10 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation. The manufacturer recommends the following isolation kits:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleid Acid Extraction System

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 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	2,5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 10 µl of the DNA sample or 10 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl.
It is necessary to keep all components at +2°C to +8°C during the PCR preparation.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
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 or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data collection	Cycles
1. Hold	37 °C	2 min		1
2. Hold	95 °C	10 min		1
3. PCR	95 °C	5 s	FAM+HEX	45
	60 °C	40 s		
	72 °C	20 s		

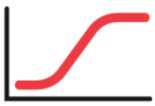
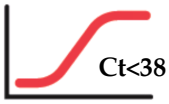
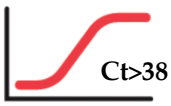
VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:

Applied Biosystems 7300/7500 Real-Time PCR System
AriaMx Real-Time PCR System
Dx/CFX96™/CFX Connect™ Real-Time PCR Detection System
LightCycler® 2.0/480
LineGene 9600
Rotor-Gene 3000, Rotor-Gene Q
SLAN® Real-Time PCR System

For detailed information about the PCR kit use with specific devices see the Manufacturer's web site or request the information from your kit supplier. If you want to use the kit with other real-time devices, contact the manufacturer.

CLINICAL SAMPLES ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation	
		**Valid**	Adenovirus	**positive**
		**Valid**	Adenovirus	**positive**
		**Valid**	Adenovirus	**negative**
		**Invalid**		
		**Invalid**		

QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the virus concentration in copies/ml while taking into account the volume of material entering the isolation:

$$\text{copy/ml} = \frac{SC \times EV}{IV}$$

SC - Sample Concentration (copy/µL)
EV - Elution Volume (µl)
IV - Isolation Volume (ml)

You can use the calculator for pathogen concentration conversion at www.geneproof.com to make the calculation easier.

WARNING

A single valid package insert for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

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