GeneProof
Molecular diagnostics for your routine

GeneProof Bordetella pertussis/parapertussis 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 extraction and PCR inhibition control		
REF	**BP/ISIN/025** 25 rxn	**BP/ISIN/050** 50 rxn	**BP/ISIN/100** 100 rxn	**BP/ISEX/025** 25 rxn	**BP/ISEX/050** 50 rxn	**BP/ISEX/100** 100 rxn
MasterMix *Bordetella*	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Positive Control *Bordetella*	1x200 µl	1x200 µl	2x200 µl	1x200 µl	1x200 µl	2x200 µl
Internal Standard *Bordetella* *Chlamydia pneumoniae* *Mycobacterium tuberculosis* *Mycoplasma pneumoniae*	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 15 repeated freezing/thawing cycles.

TECHNICAL SPECIFICATION

Target Sequence	The multi-copy insertion sequences IS1002 (specific for both Bordetella pertussis/parapertussis) and IS1001 (specific only for B. parapertussis)
Specificity	*B. pertussis*, 100 % *B. parapertussis*, 100 %
Sensitivity (LoD)	Reaches up to 0.212 cp/µl with the probability 95 % (on Amplirun® Bordetella pertussis DNA control, Vircell)
Validated Specimen	Aspirate, sputum, swab
Quality Control	In accordance with ISO 13485, each lot of GeneProof PCR Kit is tested against predetermined specifications to ensure consistent product quality.
External Quality Assessment	Regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

METHOD PRINCIPLES

The PCR kit is designed for *Bordetella pertussis* and *Bordetella parapertussis* detection and differentiation by the real-time Polymerase Chain Reaction (PCR) method. The *B. pertussis* and *B. parapertussis* detection and differentiation consists in amplification of the multi-copy insertion sequences IS1002 (specific for both *Bordetella pertussis/parapertussis*) and IS1001 (specific only for *B. parapertussis*) and in measurement of fluorescence increase. GeneProof Bordetella pertussis/parapertussis PCR Kit is one of the few kits in the market that does not provide false positive results for *Bordetella holmesii*. The *B. pertussis* presence is indicated by the FAM fluorophore fluorescence growth only. The *B. parapertussis* presence is indicated by the fluorescence growth in both FAM and Cy5 channels. An Internal Standard (IS) is either included in the reaction mix, controlling the possible inhibition of the PCR (ISIN version) or excluded, controlling also the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. 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 is designed for *in vitro* diagnostics and provides qualitative detection.

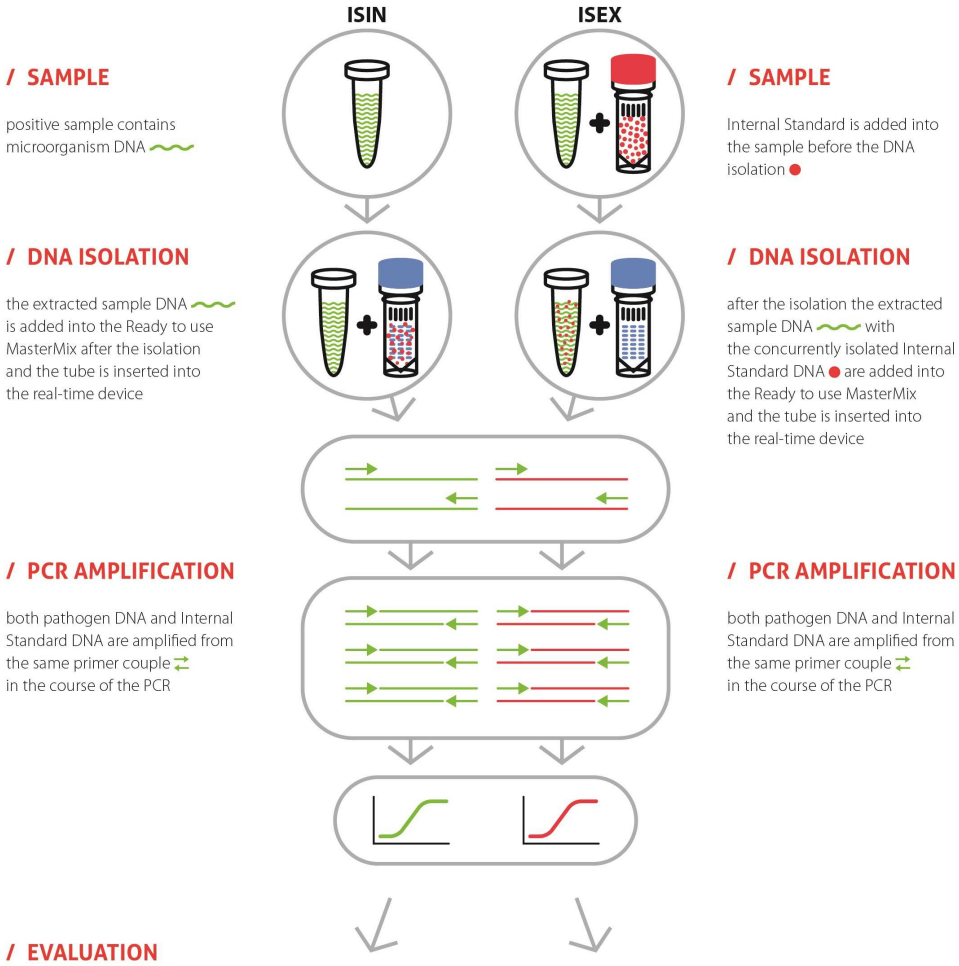
ISIN version

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.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Bordetella detection in human clinical diagnostics is feasible from aspirate, sputum and swab. Sampling of all sample types should be performed into sterile tubes without any transportation media and the samples should be transported within 24 hours at +2 °C to 8 °C. In case of longer storage all samples should be frozen below -10 °C.

NUCLEIC ACID PURIFICATION

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

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleic 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 isolated nucleic acid 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
Hold	37 °C	2 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s	FAM+HEX+Cy5	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:

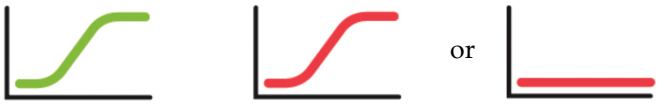
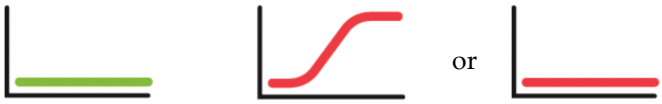
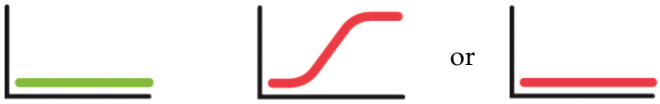
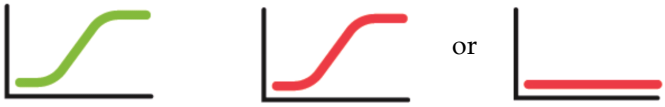
croBEE Real-Time PCR System
AriaMx Real-Time PCR System
CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 480
LineGene 9600 / 9600 Plus
Rotor-Gene 3000 / Q
SLAN® Real-Time PCR System

Required Detection Channels FAM, HEX, Cy5
GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.

CLINICAL SAMPLES ANALYSIS EVALUATION

Channel FAM	Channel Cy5	Channel HEX	Result	Interpretation
			Valid	*Bordetella pertussis* positive
			Valid	*B. parapertussis* positive
			Valid	*Bordetella parapertussis* positive
		Ct<38	Valid	negative
		Ct>38	Invalid	
			Invalid	

CONTROL ANALYSIS EVALUATION

	Channel FAM+Cy5	Channel HEX	Result
Positive Control			Valid
Negative Control			Valid
Positive Control			Invalid
Negative Control			Invalid

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.

Customer care and technical support
Tel.: +420543211679
Fax: +420516770824
email: support@geneproof.com

Orders
Tel.: +420543211679
Fax: +420516770824
email: sales@geneproof.com