Package insert

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 isolation 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 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

Target sequence	conservative region of the 1001 and 1002 insertion sequences (IS1001 and IS1002)
Detection specificity	*Bordetella pertussis* (IS1001+IS1002) and *Bordetella parapertussis* (IS1001) does not provide false positive results for *Bordetella holmesii*
Sensitivity (LOD)	for Bordetella pertussis reaches 0.02 genome/µl with the probability of 95 % for Bordetella parapertussis reaches 0.01 genome/µl with the probability of 95 %
Sample types	sputum, nasal aspirate, nasopharyngeal aspirate, bronchoalveolar lavage
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

METHOD PRINCIPLES

The PCR kit is designed for the detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* by the Polymerase Chain Reaction (PCR) method. *Bordetella* detection and differentiation is based on the amplification of the multi-copy insertion sequence IS1002 (specific for both *Bordetella* species) and the multi-copy insertion sequence IS1001 (specific only for *B. parapertussis*) and measuring the growth in the amplification product concentration using the PCR process and fluorescence labelled probes. One of the few kits on the market that do not provide false positive results for *Bordetella holmesii*. *B. pertussis* presence is indicated by the FAM fluorophore fluorescence growth and at the same time by the absence of fluorescence in the Cy5 channel. *B. parapertussis* presence is indicated by the fluorescence growth in FAM and Cy5 channels. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction (ISIN version) and possibly also the DNA extraction process 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 reaction by amplification products. The kit performs very sensitive *Bordetella* detection in clinical material (sputum, nasal aspirate, nasopharyngeal aspirate, bronchoalveolar lavage). The kit is designed for *in vitro* diagnostics and provides qualitative detection.

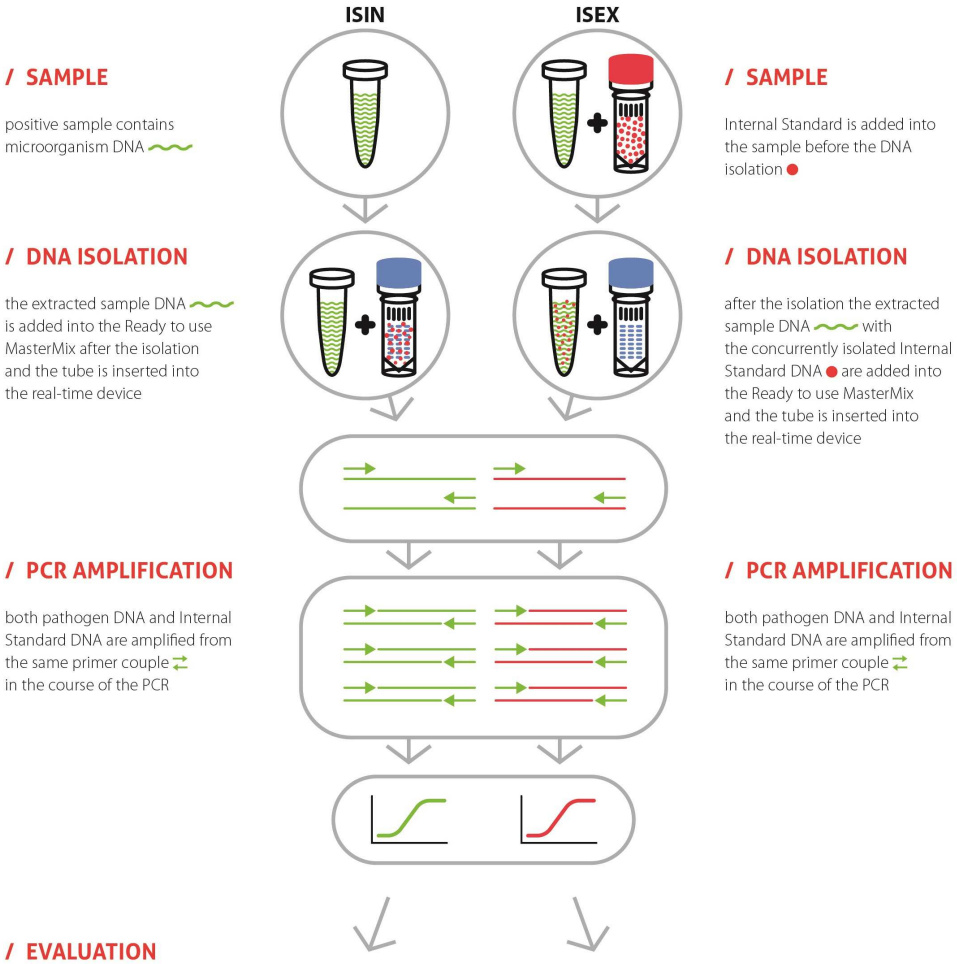
ISIN version

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 throat washings, nasopharyngeal aspirates and bronchoalveolar lavage. Sampling of all sample types should be performed into sterile tubes without any transportation media and the samples should be transported within 12 hours at +2 °C to 8 °C. In case of longer storage all samples should be frozen at -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 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
1. Hold	37 °C	2 min		1
2. Hold	95 °C	10 min		1
3. 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:

Applied Biosystems 7500 Real-Time PCR System
AriaMx Real-Time PCR System
Dx/CFX96™ Real-Time PCR Detection System
LightCycler ® 480
LineGene 9600*
Mx3000P/3005P QPCR System
Rotor-Gene 3000*, Rotor-Gene Q*
SLAN® Real-Time PCR System

* Validation applies to a device model providing detection in the following channels: FAM, HEX and Cy5.
GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.