Bordetella R-gene™ - ref.: 69-011

ARGENE

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EXTRACTION

- IN EXTRACTION ROOM -

- Schedule a pre-treatment for mucous samples.
- Homogenize **IC2** and samples to be analysed:

Respiratory specimens

- Add 10 µL **IC2** to the appropriate volume of each sample to be extracted in 1 extraction tube.
- Add 10 µL **IC2** to the appropriate volume of **W0** in 1 extraction tube.
- Perform extraction as below:

INSTRUMENTS	KIT	SAMPLE + IC2 VOLUMES	PROTOCOL	ELUTION VOLUME
	QIAamp® DNA Blood Mini Kit	200 µL of sample + 10 µL IC2		100 µL
	High Pure PCR Template Preparation Kit		Bacteria and Yeast	200 µL
QIAcube	QIAamp® DNA Blood Mini Kit		Blood and body fluid spin protocol V3	100 µL
MagNA Pure Compact®	MagNA Pure Compact Nucleic Acid Isolation Kit I		Total_NA_Plasma _100_400	
MagNA Pure LC System®	MagNA Pure DNA Isolation Kit I		DNA I Blood_Cell High Performance	
NucliSENS® easyMAG™	NucliSENS® Magnetic extraction reagents		Specific A 1.0.2	

Store extracted samples and reagents at -18°C/-22°C

ANCILLARY REAGENTS:

- **CELL Control r-gene™ - ref.: 71-106**
- **Colour Compensation r-gene™ - ref.: 71-103**

for LightCycler® 2.0 Instruments use only.

GLOSSARY:

IC2	Internal Control 2
R11	Bordetella Amplification Premix
CT	Crossing Threshold
W0	Water for extraction
IC2sample	Sample Extraction + Inhibition Control
IC2W0	Reference Extraction + Inhibition Control

AMPLIFICATION

- IN AMPLIFICATION ROOM -

Validated on:

LightCycler® 530 & 560 | SmartCycler® FCTC / FAM & Cy3 | Applied Biosystems FAM & VIC | Rotor-Gene™ GREEN & YELLOW

AMPLIFICATION PROGRAM

- Enter the following program:

STEPS		TIME	TEMPERATURE	CYCLES	FLUORESCENCE ACQUISITION
Hot Start Taq Polymerase Activation		15 min.	95°C	1	-
Amplification	Denaturation	10 sec.	95°C	45	-
	Annealing Elongation	40 sec.	60°C		530 + 560 nm end of the elongation

Note 1: for LightCycler® instruments, set **SEEK TEMPERATURE** to the temperature corresponding to the fluorescence acquisition.
Note 2: for LightCycler® instruments, add a cooling step: 30 sec. / 40°C / 1 cycle at the end of the PCR.
Note 3: for Applied Biosystems instruments, select **NONE** in **PASSIVE REFERENCE**.
Note 4: for Rotor-Gene™, calibrate the signal by clicking on **GAIN OPTIMISATION**.

- Targeted sequence is located in IS481 region. This reagent detects specifically ***Bordetella pertussis*** and other strains of *Bordetella* containing IS481 region.

AMPLIFICATION PREPARATION

- Plan number of amplification tubes taking into account:
  - ⇨ number of samples to be amplified;
  - ⇨ positive control (PC11);
  - ⇨ negative / inhibition control (**W0**);
- Distribute 15 µL R11 in all amplification tubes.
- Add 10 µL extracted sample, extracted **W0** or PC11.
- Centrifuge for 15 sec.

RESULTS

INTERPRETATION

Conditions for test validation:

- Negative Control: NO SIGNAL.
- IC2W0 (560 nm): CT ≤ 32 CYCLES.
- PC11 (530 nm): 25 ≤ CT ≤ 33 CYCLES.

Sample result interpretation

EXTRACTION and INHIBITION CONTROL	CT [IC2sample] ≤ CT [IC2W0] + 3 cycles		CT [IC2sample] > CT [IC2W0] + 3 cycles	
	NON INHIBITED and correctly extracted sample		INHIBITED or badly extracted sample	
SAMPLE	Calculated CT	Non calculated CT	Calculated CT	Non calculated CT
BORDETELLA detection	Sample validated as positive	Sample validated as negative	Sample validated as positive	NOT VALID

This outlined procedure does not replace the original protocol.

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PRINCIPLE for *Bordetella* DETECTION