ARGENE

Respiratory Multi Well System r-gene®

Influenza A/B r-gene® - ref.: 71-040 • RSV/hMPV r-gene® - ref.: 71-041
Rhino&EV/Cc r-gene® - ref.: 71-042 • AdV/hBoV r-gene® - ref.: 71-043
Chla/Myco pneumo r-gene® - ref.: 71-044 • HCoV/HPIV r-gene® - ref.: 71-045
Legio pneumo/Cc r-gene® - ref.: 71-046

EXTRACTION

- IN EXTRACTION ROOM -

Respiratory samples* : nasal washes, nasal swabs, nasopharyngeal aspirates, bronchoalveolar liquid (BAL), sputum, tracheobronchial aspirate fluid

• *Refer to the technical datasheet of each product to check which respiratory samples are validated

Perform extraction as indicated below :

INSTRUMENTS	KIT	SAMPLE VOLUME	SAMPLE TYPE	PROTOCOL	ELUTION VOLUME
NucliSENS® easyMAG® (1) (bioMérieux)	NucliSENS® easyMAG® Reagents	200 µL	Respiratory samples	Specific B + 50 µL de silice	50 µL
		400 µL			100 µL
MagNAPure Compact (2) (Roche Diagnostics)	MagNAPure Compact Nucleic acid Isolation Kit I	200 µL		Total_NA_plasma _100_400	50 µL
		400 µL			100 µL
QIAsymphony SP (3) (Qiagen)	QIAsymphony Virus/Bacteria Mini Kit	300 µL		Pathogen Complex 200	85 µL

(1) A proteinase K pre-treatment prior to extraction on the NucliSENS® easyMAG® instrument may be required if a sample is considered to be too mucous. In this case, add 10 µL for 200 µL and 20 µL for 400 µL of proteinase K at 20 mg/mL and leave to incubate for 15 minutes at 56°C.

(2) For Legio pneumo/Cc r-gene®, a pre-treatment prior to extraction on the MagNAPure Compact instrument may be required if a sample is considered to be too mucous. In this case, only use 200 µL of sample, add 20 µL of proteinase K at 20 mg/mL (proteinase K, recombinant PCR grade Roche Diagnostics, ref.: 03 115 887 001) and 180 µL of MagNAPure Bacteria Lysis Buffer (Roche Diagnostics ref.: 04 659 180 001). Incubate for 30 minutes at 63°C. Extract the totality, i.e. 400 µL (sample volume to be specified on the instrument), the elution is performed in 50 µL.

(3) Not CE-marked with Legio pneumo/Cc r-gene®

ANCILLARY REAGENTS:

- **CELL Control r-gene® - ref.: 71-106 kit to check the presence of cells in the sample.**
- **DICO Extra r-gene® - ref.: 71-101**
- **RICO Extra r-gene® - ref.: 71-105**

GLOSSARY:

PC4X	Positive Control
W0	Negative Extraction + Amplification Control
R4X	Amplification Premix
CT	Crossing Threshold
RT	Reverse Transcriptase
S	Sample

AMPLIFICATION

- IN AMPLIFICATION ROOM -

LightCycler 480	Applied Biosystems 7500 Fast,7500 Fast Dx, StepOne, ViiA7	Stratagene Mx3005P Agilent Mx3005P Versant kPCR AD	Dx Real-Time System (Bio-Rad)	Rotor-Gene Q
530 & 560	FAM & VIC	FAM & HEX	FAM & HEX	GREEN & YELLOW

Refer to the technical datasheet of each product to check which amplification platforms are validated

AMPLIFICATION PROGRAM

STEPS		TIME	TEMPERATURE	CYCLES	FLUORESCENCE ACQUISITION
Reverse Transcription		5 min.	50°C	1	-
Taq Polymerase Activation		15 min.	95°C	1	-
Amplification	Denaturation	10 sec.	95°C	45	-
	Annealing	40 sec.	60°C		530 + 560 nm
	Elongation	25 sec.	72°C		-

Note 1: the temperature variations are parametered by default, which means at 100% or at their maxima.
Note 2: on LightCycler 480, only "System II" is compatible with the use of the kits. The "System II" features an automatically activated colour compensation inits software.
Note 3: on Applied Biosystems 7500 Fast, 7500 Fast Dx, ViiA7, select **ROX** as **PASSIVE REFERENCE** when programming.
Note 4: on Applied Biosystems StepOne **only**, select **NONE** as **PASSIVE REFERENCE** when programming.
Note 5: on Rotor-Gene Q, calibrate the signal by clicking on **GAIN OPTIMISATION**.
Note 6: on Stratagene Mx3005P or Agilent Mx3005P or Versant kPCR Molecular System AD, select **NONE** in **REFERENCE DYE** when programming.

AMPLIFICATION PREPARATION

Preparation of the premix

- Plan "**n**" wells for amplification taking into account:
  number of samples to be amplified;
  number of Positive Control (**PC4X**);
  number of Negative Extraction + Amplification Control (**W0**);

Note:for Dx Real-Time System, use transparent plates (ref.: HSP9601) with optical caps (ref.: TCS0803).

Step to be added for RNA premix only

- Pipet **15 µL x (n+1)** AmplificationPremix (**R4X**);
- Dilute **1 volume** RT (1 µL) into **9 volumes H_2O** (9 µL);
- Add **0.15 µL x (n+1)** diluted Reverse Transcriptase.

Preparation of PCR assay

- For RNA detection: Distribute **15 µL** of the previous premix (**R4X** + **diluted RT**) in the amplification wells;

or

- For DNA detection: Distribute **15 µL** of amplification premix (**R4X**) in the amplification wells.
- Add **10 µL** of extracted sample, positive control (**PC4X**), extracted **W0**.
- Centrifuge for 15 sec, if applicable.
- Place the plate in the instrument and run the program described above according to the protocol of the instrument used.

RESULTS

TEST VALIDATION CONDITIONS

- **W0** read at **530** nm and **560** nm: **NO SIGNAL**.
- **PC4X** read at **530** nm and **560** nm: **CT < 32 CYCLES**.

INTERPRETATION OF THE RESULTS

RESULTS		Target A - 530 nm: CT calculated < 40 cycles	Target A - 530 nm: No CT calculated or CT ≥ 40 cycles
Target B - 560 nm	CT calculated < 40 cycles	**Target A & B*** +	**Target A** - **Target B** + if cellular control validated
	No CT calculated or CT ≥ 40 cycles	**Target A** + **Target B** - if cellular control validated	**Target A & B** - if cellular control validated

*For Applied Biosystems StepOne and Rotor-Gene Q apparatus, a crosstalk from **530** nm into **560** nm may be observed.

Note: The evaluation of the efficacy of the extraction and the detection of potential inhibitors is possible using the DICO Extra r-gene® ref.: 71-101 / RICO Extra r-gene® ref.: 71-105 reagents.

Note: Certain batches of transport media used for routing and storage of respiratory samples may contain traces of genomic DNA likely to give a false positive result beyond 35 cycles*. For the same reasons, the use of inhibition controls IC1 (RICO Extra r-gene®) or IC2 (DICO Extra r-gene®) in combination with this cellular control may also engender weakly positive signals beyond 35 cycles. In both cases, **any sample with a CT value for the cellular control target greater than 35 cycles is considered to be a sample with an insufficient number of cells to be interpreted as valid**. This sample must be retested or taken again.

**at 560 nm (cell control channel) for kits containing cellular control (Rhino&EV/Cc r-gene® ref.: 71-042 and Legio pneumo/Cc r-gene® ref.: 71-046)*

This outlined procedure does not replace the original protocol
See Package Insert

Reference 71-040 – 71-040CE _ v2 *Reference 71-044 – 71-044CE _v2*
Reference 71-041 – 71-041CE _ v2 *Reference 71-045 – 71-045CE _v2*
Reference 71-042 – 71-042CE _ v3 *Reference 71-046 – 20682 – A.*
Reference 71-043 – 71-043CE _ v2

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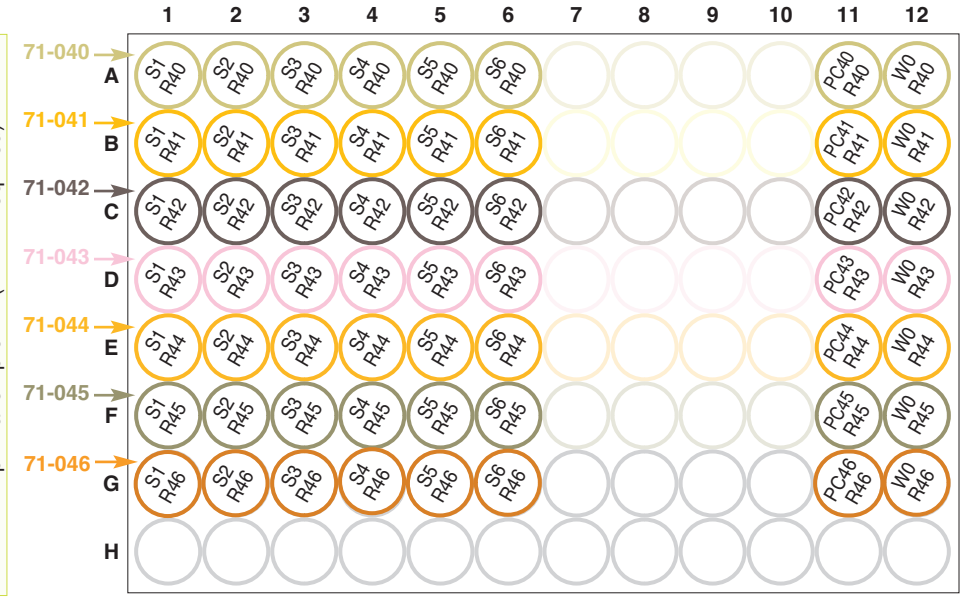
Principle for Respiratory Multi Well System r-gene® detection

DISTRIBUTION

Example of microplate layout

DISTRIBUTE SAMPLES AND CONTROLS (10 µL PER WELL)

Distribute mix (15 µL per well):
Amplification premix (+ RT if required)



VALIDATION AND INTERPRETATION OF THE RESULTS

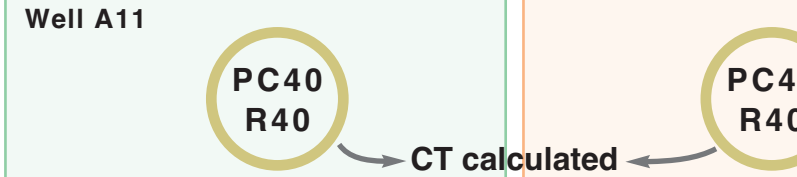
e.g.: Detection with the *Influenza* A/B r-gene® - 71-040 kit.

530 nm reading	560 nm reading

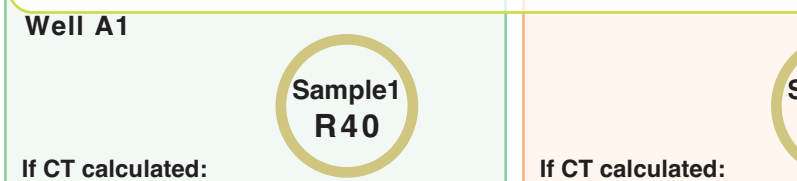
TEST VALIDATION CONDITIONS

1st condition: **N**egative **E**xtraction + **A**mplification **C**ontrol (**W0**)

2nd condition: **P**ositive **C**ontrol (**PC40**)



INTERPRETATION OF THE RESULTS



If CT calculated:
-> Sample1 Positive for *Influenza* A
If No CT calculated or CT>40 cycles:
-> Sample1 Negative for *Influenza* A
if cellular control validated

If CT calculated:
-> Sample1 Positive for *Influenza* B
If No CT calculated or CT>40 cycles:
-> Sample1 Negative for *Influenza* B
if cellular control validated

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This outlined procedure does not replace the original protocol
See Package Insert

Reference 71-040 – 71-040CE _ v2 *Reference 71-044 – 71-044CE _ v2*
Reference 71-041 – 71-041CE _ v2 *Reference 71-045 – 71-045CE _ v2*
Reference 71-042 – 71-042CE _ v3 *Reference 71-046 – 20682 – A.*
Reference 71-043 – 71-043CE _ v2