PERFORMANCES CONFIRMATION HANDBOOK

Bio-T kit® PRRSV DIVA

Cat. N° BIOTK077 - 50 reactions
Cat. N° BIOTK086 - 100 reactions

Handbook for titrated nucleic acids and Process Positive Control (MRI) to confirm the performances of the Bio-T kit® PRRSV DIVA

PRRSV EU other than Suvaxyn® RNA - Cat. N° cARNPRRSVEU-003
Suvaxyn® vaccine strain RNA - Cat. N° cARNPRRSVVAC001

SWINE

Sample Types

- Whole blood (on EDTA), serum
- Oral Fluids
- Organs
- Individual analysis or by pool up to 10 according to the matrix

Recommended nucleic acids (NA) extractions

- Magnetic beads extraction (e.g.: BioSellal – BioExtract® SuperBall® Cat. N° BES384)
- Silica membrane columns extraction (e.g.: BioSellal – BioExtract® Column Cat. N° BEC050 or BEC250)

Veterinary use only

DOCUMENTS MANAGEMENT

This handbook aims to explain how to monitor the performances of your thermal cycler(s). It also aims to detail the use of the process positive control (MRI) to monitor the performances of your complete method over the time. Thus, for the qRT-PCR protocol, please refer to the Bio-T kit® PRRSV DIVA qRT-PCR handbook, presenting the instruction information to perform the qRT-PCR.
The last versions in use for each handbook are indicated on the certificate of analysis (CA) provided with the Bio-T kit® PRRSV DIVA.

Besides these two handbooks, a summary report of the validation file is available on request, contact BioSellal (contact@biosellal.com).

MODIFICATIONS MANAGEMENT

BioSellal indicates modifications done to this document by highlighting them using the rules presented in the Table below:

MODIFICATIONS MANAGEMENT			
Type of modification Highlighting color	**Minor modifications**	**Type 1 Major modifications**	**Type 2 Major modifications**
Impact on revision / version	**Change of revision date** **No change of version**	**Change of revision date** **+ change of version**	**Change of revision date** **+ change of version**
Examples of modifications	Corrections: typographical, grammatical or turns of phrase	EPC reference modification	Modification of Master Mix composition
	Addition of new sample type for extraction	Exogenous IPC reference modification	Modification of validated extraction protocol
	Addition of information giving more details or alternative protocol		

PRESENTATION

Performances confirmation of the Bio-T kit® PRRSV DIVA

Bio-T kit® PRRSV DIVA (Cat. N° BIOTK077/BIOTK086) allows a distinction between European strains of PRRSV other than Suvaxyn® (PRRSV EU other than Suvaxyn®) and Suvaxyn® PRRS MLV vaccine strain (Suvaxyn® vaccine strain) from whole blood (on EDTA), serum, oral fluids and organs. This kit was developed and validated according to the **French regulatory standard NF U47-600-2 edited by AFNOR.**
The pool up to 10 is possible for whole blood (on EDTA) and serum matrices with the Bio-T kit® PRRSV DIVA.

This handbook details the steps required to confirm performances of your thermal cycler(s) and the use of the Process Positive Control (MRI). BioSellal provides the Process Positive Control (MRI, Cat. N° MRI-PRRSV-001), that is common for Bio-T kit® PRRSV DIVA and Bio-T kit® PRRSV.

BioSellal provides synthetic RNA of PRRSV EU other than Suvaxyn® and Suvaxyn® vaccine strain (titrated in number of copies/qRT-PCR) to confirm performances of your thermal cycler(s).Please, contact BioSellal for more information (tech@biosellal.com).

List of reagents to confirm laboratory performances and other consumables required

Reagents to confirm laboratory performances and consumables*			
Reagent	**Description**	**Provider**	**Cat. N°**
PRRSV EU other than Suvaxyn® RNA	Quantified RNA of PRRSV EU other than Suvaxyn® (1.2×10^5 copies/qRT-PCR)	BioSellal	cARNPRRSVEU-003
Suvaxyn® vaccine strain RNA	Quantified RNA of Suvaxyn® vaccine strain (1.2×10^5 copies/qRT-PCR)	BioSellal	cARNPRRSVVAC001
Serum MRI	Positive serum for PRRSV EU other than Suvaxyn®	BioSellal	MRI-PRRSV-001
ATL Buffer	Lysis Buffer	BioSellal	ATL19076
BioExtract® Column	DNA/RNA column extraction kit (50)	BioSellal	BEC050
BioExtract® Column	DNA/RNA column extraction kit (250)	BioSellal	BEC250
BioExtract® SuperBall®	DNA/RNA Magnetic beads extraction kit (4 x 96)	BioSellal	BES384
Bio-T kit® PRRSV DIVA	Distinction between PRRSV EU other than Suvaxyn® and Suvaxyn® vaccine strain with exogenous IPC	BioSellal	BIOTK077 (50 reactions)
			BIOTK086 (100 reactions)

* These reagents are available only on demand, please contact BioSellal (contact@biosellal.com).
For consumables related to the thermal cycler, refer to the user manual of the device.

Main critical points

- Wear appropriate personal protective equipment (lab coat, disposable gloves frequently changed).
- Work in dedicated and separate areas to avoid contamination: "Extraction" (unextracted samples storage, extraction equipment area), "Mix" (ready to use MM storage, qRT-PCR plates preparation), "Nucleic acids (NA) Addition" (Nucleic Acids storage and addition of extracted NA and controls in the qRT-PCR plate), "PCR" (final area containing the thermal cycler(s)).
- Use dedicated equipment for each working area (gloves, lab coat, pipettes, vortex, ...).
- Use filter tips.
- Before use, thaw all components at room temperature.
- **One-step RT-PCR Master-Mix is less stable than PCR Master-Mix. To guarantee its optimal performance, it is mandatory to extemporaneously defrost the tubes just before the use, to vortex it, to keep it at 5°C ± 3 during the deposit and to refreeze it immediately afterwards.**
- Vortex and spin briefly (mini-centrifuge) all reagents before use.
- Avoid the repetition of freezing-thawing cycles for samples, lysates, extracted NA.
- **Pathogen's genome detected by the PIG line's kits can be DNA or RNA. Working with RNA is more demanding than working with DNA** (RNA instability and omnipresence of the RNases). For these reasons, special precautions must be taken:
    - Always wear gloves, change them frequently, especially after contact with skin or work surfaces.
    - Treat all surfaces and equipment with RNases inactivation agents (available commercially).
    - When wearing gloves and after material decontamination, minimize the contact with surfaces and equipment in order to avoid the reintroduction of RNases.
    - Use "RNase free" consumable.
    - It is recommended to store the RNA at ≤ 5 ±3°C during the manipulation and then freeze it as soon as possible, preferably at ≤ -65°C or by default at ≤ -16°C.
    - Open and close tubes one by one in order to limit the opening times and avoid any contact with RNases present in the environment (skin, dust, working surfaces…).

PERFORMANCES CONFIRMATION OF BIO-T KIT® PRRSV DIVA

Confirmation of your thermal cycler performances

General principle

This step allows the confirmation that your devices are able to reproduce the qRT-PCR performance described in the summary of the validation file.
As Bio-T kit® PRRSV DIVA allows a distinction between PRRSV EU other than Suvaxyn® and Suvaxyn® vaccine strain, BioSellal recommends to verify qRT-PCR detectability using a target concentration at 3 time the $LD_{RT\text{-}PCR}$ (3 x $LD_{RT\text{-}PCR}$). BioSellal recommends also to verify the PCR efficiency.

To be noted: The data obtained by BioSellal during the validation of the Bio-T kit® PRRSV DIVA demonstrated a phenomenon of competition between targets. Indeed, when a sample is strongly positive for one of the two targets, the detection sensitivity of the other target is impacted. Also, in the case of a highly positive result for PRRSV EU strains other than Suvaxyn®, there is a risk of not detecting a small amount of Suvaxyn® vaccine strain. The negative result for the vaccine strain Suvaxyn® channel is therefore to be interpreted according to the vaccination status of the animals (date and vaccination protocol, date of sampling ...). Conversely, in the case of a highly positive result for the Suvaxyn® vaccine strain, there is a risk of non-detection of a PRRSV EU strains other than Suvaxyn® in a small quantity. The negative result for the PRRSV EU other than Suvaxyn® channel is therefore to be interpreted according to the epidemiological context (date of vaccination, viral circulation ...). It is possible to check the presence of the Suvaxyn® vaccine strain in combination with a PRRSV EU strain other than Suvaxyn® by Next-Generation Sequencing. Please contact BioSellal for more information. It may also be possible to renew the sampling later after the date of vaccination.
Thus, BioSellal established also the $LD_{RT\text{-}PCR}$ for each target without competition.

Confirmation of 3 x $LD_{RT\text{-}PCR}$

The Bio-T kit® PRRSV DIVA is a triplex, thus, according to the **French regulatory standard NF U47-600-2 edited by AFNOR,** $LD_{RT\text{-}PCR}$ was established in the worst condition, that means in competition with the other target in excess. Each $LD_{RT\text{-}PCR}$ is described in the summary of the validation file of the Bio-T kit® PRRSV DIVA. However, a phenomenon of competition between the targets PRRSV EU other than Suvaxyn® and Suvaxyn® vaccine strain has been identified. Thus, BioSellal established also the $LD_{RT\text{-}PCR}$ for each target without competition.

Summary of LD_{RT-PCR} obtained:

Target	with/without competition	Number of copies GE /RT-PCR
PRRSV EU other than Suvaxyn®	With	4 000
	Without	40
Suvaxyn® vaccine strain	With	400
	Without	20

Experimental design

Number of run	Number of operators	Number of wells with 3 x LD_{RT-PCR} level per run
1 per thermal cycler 2 if only one thermal cycler	1	At least 2 Wells / Peltier block

Plate preparation

BioSellal provides synthetic RNA of PRRSV EU other than Suvaxyn® and of Suvaxyn® vaccine strain titrated in number of copies/qRT-PCR to confirm performances of your thermal cycler(s) (Cat. N°cARNPRRSVEU-003, cARNPRRSVVAC001).
PRRSV EU other than Suvaxyn® RNA is provided at 1.2 x 10^5 copies /qRT-PCR.
Suvaxyn® vaccine strain RNA is provided at 1.2 x 10^5 copies /qRT-PCR.

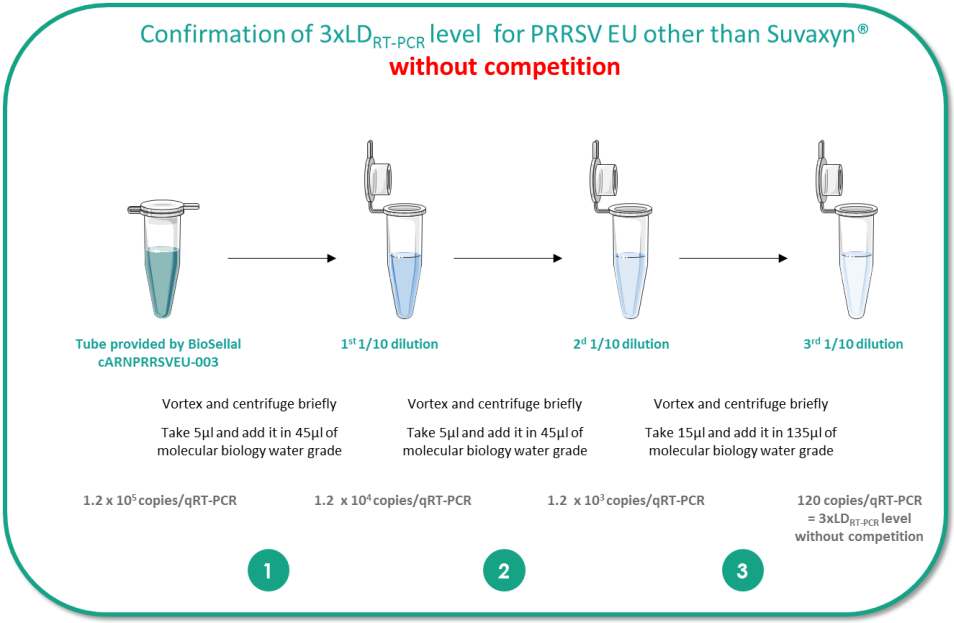
⚠ CAUTION: *PRRSV EU RNA and Suvaxyn® vaccine strain RNA tube handling represents nucleic acids contamination hazard, it is thus recommended to open and handle it in a restricted area, away from other PCR components and to take precautions to avoid cross-contamination with nucleic acids extracts during deposit on the qRT-PCR plate.*

Working with RNA is more demanding than working with DNA (RNA instability and omnipresence of the RNases). For these reasons, BioSellal recommends applying the precautions indicated on page 4.

Before use, defrost the RNA, vortex and centrifuge briefly before diluting:
- **PRRSV EU other than Suvaxyn® RNA is provided at 1.2 x 10^5 copies /qRT-PCR corresponding to 3000 times the level of LD_{RT-PCR} without competition.**

To reach the 3 x LD_{RT-PCR} level without competition:

Carry out serial dilution in molecular biology water (RNase/DNase free) or 1X TE to reach the 3 x LD_{RT-PCR} level: 3 x 40 = 120 number of copies/qRT-PCR corresponding to a global dilution of 1/1000. Between each dilution, thoroughly vortex and centrifuge (benchtop centrifuge) to homogenize.

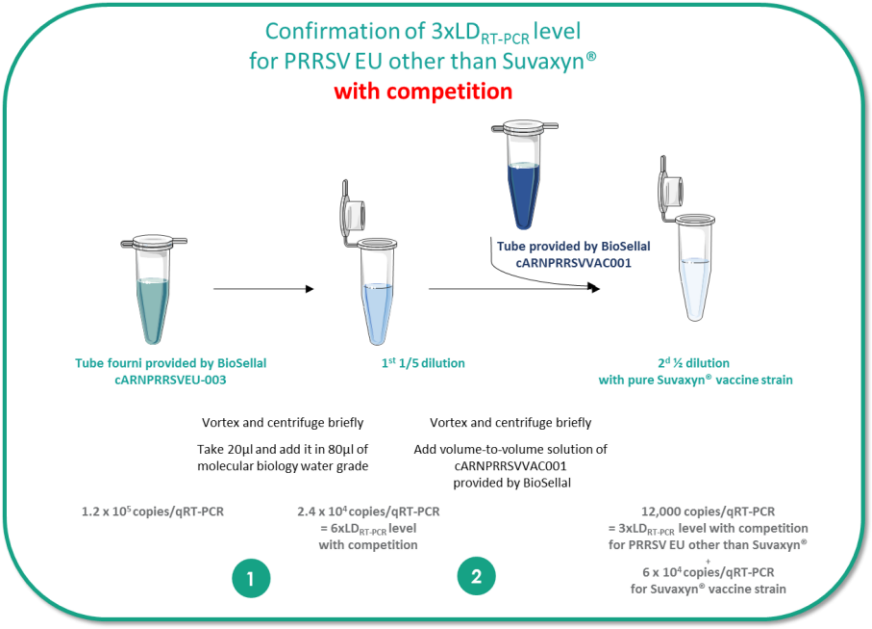


The resulting solution contains 120 number of copies/qRT-PCR of PRRSV EU other than Suvaxyn® target = 3 x LD_{RT-PCR} without competition.

To reach the 3 x LD_{RT-PCR} level with competition:

Carry out serial dilution in molecular biology water (RNase/DNase free) or 1X TE to reach the 6 x LD_{RT-PCR} level: 6 x 4,000 = 24,000 copies/qRT-PCR corresponding to a global dilution of 1/5. Between each dilution, thoroughly vortex and centrifuge (benchtop centrifuge) to homogenize.

Then it is then necessary to add volume-to-volume solution of pure Suvaxyn® vaccine strain, to validate the 3xLD_{RT-PCR} in the worse conditions meaning when the other target is in excess (Ct ~ 23).

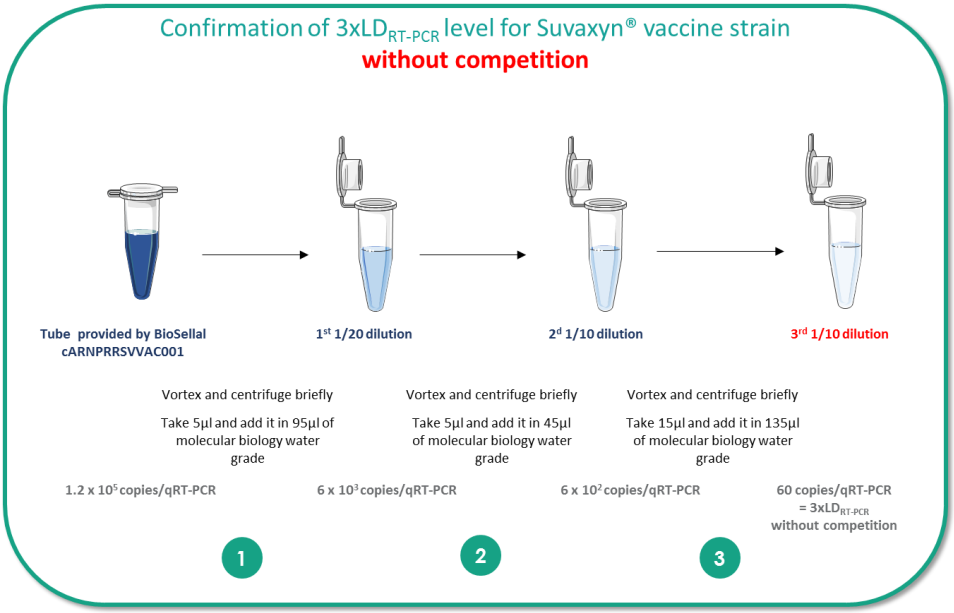


The resulting solution contains 12,000 GE/qRT-PCR of PRRSV EU other than Suvaxyn® target (3 x LD_{RT-PCR}) and 6 x 10^4 GE/qRT-PCR of Suvaxyn® vaccine strain target (expected Ct value ~ 23).

- **Suvaxyn® vaccine strain RNA is provided at 1.2 x 10^5 number of copies /qRT-PCR corresponding to 6,000 times the level of LD_{RT-PCR} without competition.**

To reach the 3 x LD_{RT-PCR} level without competition:

Carry out serial dilution in molecular biology water (RNase/DNase free) or 1X TE to reach the 3 x LD_{RT-PCR} level: 3 x 20 = 60 number of copies/qRT-PCR corresponding to a global dilution of 1/2000. Between each dilution, thoroughly vortex and centrifuge (benchtop centrifuge) to homogenize.

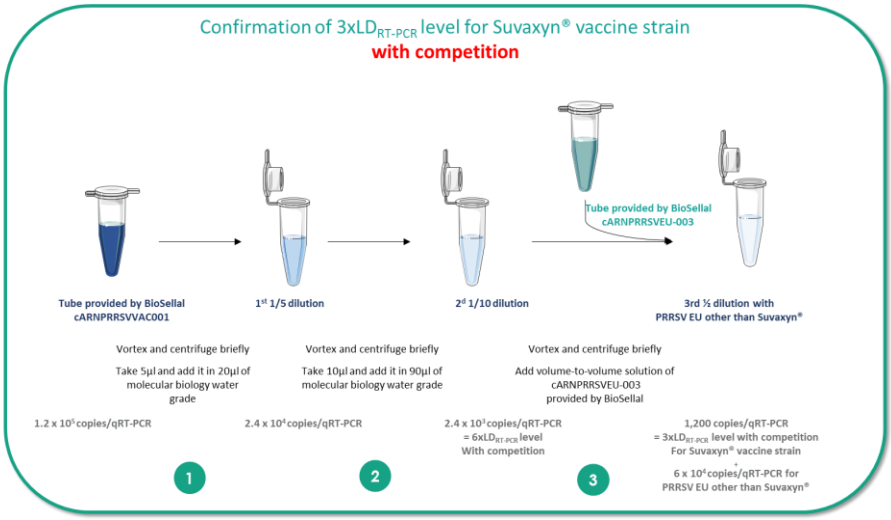


The resulting solution contains 60 number of copies/qRT-PCR of vaccine strain target = 3 x LD_{RT-PCR} without competition.

To reach the 3 x LD_{RT-PCR} level with competition:

Carry out serial dilution in molecular biology water (RNase/DNase free) or 1X TE to reach the 6 x LD_{RT-PCR} level: 6 x 400 = 2,400 copies/qRT-PCR corresponding to a global dilution of 1/50. Between each dilution, thoroughly vortex and centrifuge (benchtop centrifuge) to homogenize.

Then it is then necessary to add volume-to-volume solution of pure PRRSV EU other than Suvaxyn®, to validate the LD_{RT-PCR} in the worse conditions meaning when the other target is in excess (Ct ~ 25).



The resulting solution contains 1,200 GE/qRT-PCR of vaccine strain target (3 x LD_{RT-PCR}) and 6 x 10^4 GE/qRT-PCR of PRRSV EU other than Suvaxyn® target (expected Ct value ~ 25).

5 µl of the 3 x LD_{RT-PCR} level must be dispense in the RT-PCR plate with 20 µl of Master Mix.

Plate setup

In order to confirm the performances of your device, it is important to verify both the thermal part (all Peltier blocks which work independently) and the optical part. To this aim, BioSellal recommends dispensing at least 2 wells per Peltier Blocks. Thus, the plate setup depends on the thermal cyclers you use. A plate setup is suggested for ABI PRISM® 7500 Fast (Applied BioSystems) and for AriaMx™ (Agilent Technologies). For other thermal cyclers, please contact BioSellal (tech@biosellal.com).

PRRSV EU other than Suvaxyn®
Suvaxyn® vaccine strain

- **For ABI PRISM® 7500 Fast (Applied BioSystems): 4 Peltier blocks, CCD camera with side effect**

	1	2	3	4	5	6	7	8	9	10	11	12
A	3xLD RT-PCR	3xLD RT-PCR									3xLD RT-PCR	3xLD RT-PCR
B												
C				3xLD RT-PCR	3xLD RT-PCR				3xLD RT-PCR	3xLD RT-PCR		
D												
E												
F				3xLD RT-PCR	3xLD RT-PCR				3xLD RT-PCR	3xLD RT-PCR		
G												
H	3xLD RT-PCR	3xLD RT-PCR									3xLD RT-PCR	3xLD RT-PCR

- **For AriaMx™ (Agilent Technologies): 6 Peltier blocks, line reading**

	1	2	3	4	5	6	7	8	9	10	11	12
A							3xLD RT-PCR	3xLD RT-PCR				
B	3xLD RT-PCR	3xLD RT-PCR									3xLD RT-PCR	3xLD RT-PCR
C			3xLD RT-PCR	3xLD RT-PCR					3xLD RT-PCR	3xLD RT-PCR		
D					3xLD RT-PCR	3xLD RT-PCR						
E					3xLD RT-PCR	3xLD RT-PCR						
F			3xLD RT-PCR	3xLD RT-PCR					3xLD RT-PCR	3xLD RT-PCR		
G	3xLD RT-PCR	3xLD RT-PCR									3xLD RT-PCR	3xLD RT-PCR
H							3xLD RT-PCR	3xLD RT-PCR				

For thermal cycler setting, please refer to the instructions detailed in the qRT-PCR handbook of the Bio-T kit® PRRSV DIVA.

Expected results

To confirm performances of your thermal cycler(s), all wells containing the 3 x $LD_{RT\text{-}PCR}$ level must be "detected" for the target of interest.
No detection is expected for exogenous IPC.
Data obtained by BioSellal for the 3 x $LD_{RT\text{-}PCR}$ level are available in the certificate of analysis provided with the titrated nucleic acids.

Confirmation of RT-PCR efficiency

The RT-PCR efficiency for each target was established in the worst condition, that means in competition with the other target in excess. Each RT-PCR efficiency is described in the summary of the validation file of the Bio-T kit® PRRSV DIVA. However, a phenomenon of competition between the targets PRRSV EU other than Suvaxyn® and Suvaxyn® vaccine strain has been identified. Thus, BioSellal established also the efficiency for each target without competition.

Summary of obtained RT-PCR efficiency without competition:

Target	With/Without competition	RT-PCR efficiency
PRRSV EU other than Suvaxyn®	With	96 %
	Without	97 %
Suvaxyn® vaccine strain	With	82 %
	Without	112 %

Experimental design

Number of run	Number of operators	Number of calibration range
1 per thermal cycler 2 if only one thermal cycler	1	1

Plate preparation

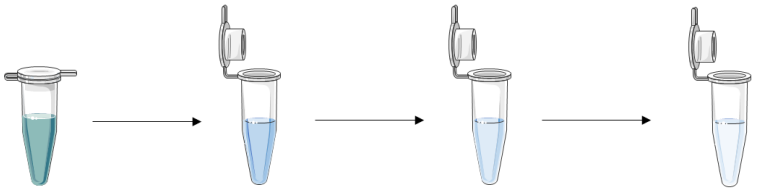
BioSellal provides synthetic RNA of PRRSV EU other than Suvaxyn® and of Suvaxyn® vaccine strain titrated in number of copies/qRT-PCR to confirm performances of your thermal cycler(s) (Cat. N°cARNPRRSVEU-003, cARNPRRSVVAC001).

PRRSV EU other than Suvaxyn® RNA is provided at 1.2 x 10^5 copies /qRT-PCR.

Suvaxyn® vaccine strain RNA is provided at 1.2 x 10^5 copies /qRT-PCR.

These concentrations do not allow the confirmation of RT-PCR efficiency with competition, so BioSellal recommends to do it without competition. Before use, defrost the RNA, vortex and centrifuge briefly before diluting.

Confirmation of RT-PCR efficiency for PRRSV EU other than Suvaxyn®
without competition



Tube provided by BioSellal cARPRRSVEU-003 | 1st 1/10 dilution | 2d 1/10 dilution | 3rd 1/10 dilution

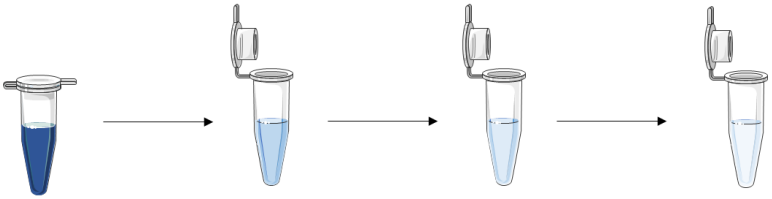
Vortex and centrifuge briefly
Take 5µl and add it in 45µl of molecular biology water grade

Vortex and centrifuge briefly
Take 5µl and add it in 45µl of molecular biology water grade

Vortex and centrifuge briefly
Take 5µl and add it in 45µl of molecular biology water grade

1.2 x 10^5 copies/qRT-PCR | 1.2 x 10^4 copies/qRT-PCR | 1.2 x 10^3 copies/qRT-PCR | 1.2 x 10^2 copies/qRT-PCR

Confirmation of RT-PCR efficiency for Suvaxyn® vaccine strain
without competition



Tube provided by BioSellal cARNPRRSVVAC001 | 1st 1/10 dilution | 2d 1/10 dilution | 3rd 1/10 dilution

Vortex and centrifuge briefly
Take 5µl and add it in 45µl of molecular biology water grade

Vortex and centrifuge briefly
Take 5µl and add it in 45µl of molecular biology water grade

Vortex and centrifuge briefly
Take 5µl and add it in 45µl of molecular biology water grade

1.2 x 10^5 copies/qRT-PCR | 1.2 x 10^4 copies/qRT-PCR | 1.2 x 10^3 copies/qRT-PCR | 1.2 x 10^2 copies/qRT-PCR

5 µl of each dilution must be dispense in the PCR plate with 20µl of Master Mix.

Plate setup

There is no obligation regarding the plate setup. For ABI PRISM® 7500 Fast (Applied BioSystems) and for AriaMx™ (Agilent Technologies) BioSellal suggests the following plate setup. For other thermal cyclers, please contact BioSellal (tech@biosellal.com).

PRRSV EU other than Suvaxyn®
Suvaxyn® vaccine strain

- **For ABI PRISM® 7500 Fast (Applied BioSystems): 4 Peltier blocks, CCD camera with side effect**

	1	2	3	4	5	6	7	8	9	10	11	12
A					1,2. 10^5 copies/ RT-PCR	1,2. 10^4 copies/ RT-PCR	1,2. 10^3 copies/ RT-PCR	1,2. 10^2 copies/ RT-PCR				
B					1,2. 10^5 copies/ RT-PCR	1,2. 10^4 copies/ RT-PCR	1,2. 10^3 copies/ RT-PCR	1,2. 10^2 copies/ RT-PCR				
C												
D												
E												
F												
G												
H												

- **For AriaMx™ (Agilent Technologies): 6 Peltier blocks, line reading**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B							1,2. 10^5 copies/ RT-PCR	1,2. 10^5 copies/ RT-PCR				
C							1,2. 10^4 copies/ RT-PCR	1,2. 10^4 copies/ RT-PCR				
D							1,2. 10^3 copies/ RT-PCR	1,2. 10^3 copies/ RT-PCR				
E							1,2. 10^2 copies/ RT-PCR	1,2. 10^2 copies/ RT-PCR				
F												
G												
H												

For thermal cycler setting, please refer to the instructions detailed in the qRT-PCR handbook of the Bio-T kit® PRRSV DIVA.

Expected results

Efficiency for each target must be comparable with those obtained by BioSellal. (Cf. Page 12)

MONOTORING OF THE BIO-T KIT® PRRSV DIVA WHOLE METHOD PERFORMANCES WITH THE PROCESS POSITIVE CONTROL (MRI)

Description

Process Positive Control (MRI) is a weak positive sample of whole blood (on EDTA), serum, oral fluids or organs positive for PRRSV EU other than Suvaxyn® and Suvaxyn® vaccine strain. It has to be extracted in parallel with tested samples. After qRT-PCR, MRI Ct values will be monitored on a Shewhart control card. Obtaining conform Ct values with MRI validates the whole process.

Experimental design

Process Positive Control (MRI) could be a positive sample with a Ct value included in the variability zone of the standard normal distribution (between Ct 30-33). Otherwise it may be produce with a negative sample supplemented by PRRSV EU other than Suvaxyn® and Suvaxyn® vaccine strain positive sample to reach the Ct of 30-33.

The determination of the average target Ct value and the standard deviations of the MRI batch can be carried out under conditions of intra-series repeatability and intermediate fidelity by varying the operating conditions (for example, the manipulator, the day, the thermal cycler, the extraction device ...).

Expected results

The prepared MRI batch must be aliquoted in single doses. During routine tests, it must be adding to each series of extraction to monitor the fidelity of the complete method.

biosellal

www.biosellal.com

Technical Support

tech@biosellal.com +33 (0) 4 26 78 47 62

Information and orders

contact@biosellal.com +33 (0) 4 26 78 47 60