EXTRACTION HANDBOOK

Nucleic acids extraction methods recommended for the use of Bio-T kit® PRRSV, PRRSV DIVA, PCV3, PCV2 & PCV3

Bio-T kit® PRRSV	Cat. N° BIOTK001 – 50 reactions	Cat. N° BIOTK040 – 100 reactions
Bio-T kit® PRRSV DIVA	Cat. N° BIOTK077 – 50 reactions	Cat. N° BIOTK086 – 100 reactions
Bio-T kit® PCV3	Cat. N° BIOTK070 – 50 reactions	Cat. N° BIOTK071 – 100 reactions
Bio-T kit® PCV2 & PCV3	Cat. N° BIOTK072 – 50 reactions	Cat. N° BOTK073 – 100 reactions

SWINE

Sample Types

- Whole blood (on EDTA), serum
- Oral Fluids
- Organs
- Cell culture supernatant
- 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

Bio-T kit® PRRSV, PRRSV DIVA, PCV3, PCV2 & PCV3 have two technical handbooks:

- The extraction handbook shared between Bio-T kit® PRRSV, PRRSV DIVA, PCV3, PCV2 & PCV3, displaying BioSellal's validated extraction protocols for each type of sample.
- For each Bio-T kit®, a specific qPCR or qRT-PCR handbook, presenting the instruction to perform the qPCR or qRT-PCR.

The last versions in use for each handbook are indicated on the certificate of analysis (CA) provided with each Bio-T kit®.

Besides these two handbooks, a summary report of the validation file and a performances confirmation handbook are available on request, contact BioSellal (contact@biosellal.com).

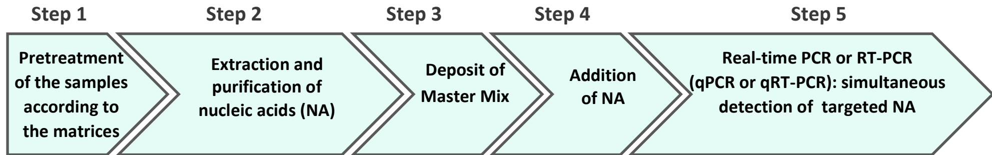
MODIFICATIONS MANAGEMENT

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

Description of the whole process



Extraction hanbook shared between the Bio-T kit® PRRSV, PRRSV DIVA, PCV3, PCV2 & PCV3		**Specific Bio-T kit® handbook (PRRSV, PRRSV DIVA, PCV3 or PCV2 & PCV3)**		
Whole blood, serum Oral Fluids* Organs* Cell culture supernatant	BioExtract® SuperBall® BioExtract® Column	Ready-to-use Master Mix	Samples NC/NCS Process Positive control (MRI or MRSI) PCR controls (EPC, artifact control for PRRSV DIVA only)	Refer to the Bio-T kit® user's handbook or the table summarizing the main qPCR parameters of the PIG line.

*pretreatment mandatory

The protocol of the qPCR or qRT-PCR part is detailed in the user's handbook of the different Bio-T kit®.

List of consumables and reagents recommended for extraction

Table 1. Consumables and reagents not provided with the kit

Consumable / Reagent	Description	Provider	Cat. N°
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 Extraction kit Magnetic beads (4 x 96)	BioSellal	BES384

List of reagents for performances validation

For Bio-T kit® PRRSV :

To confirm performances of your thermal cycler(s) or your whole process, PRRSV EU and PRRSV NA RNA transcripts (quantified in copies/qRT-PCR) and PRRSV EU and PRRSV NA viral suspension (quantified in TCID50/ml), used by BioSellal in the validation file, could be supplied. Reference material (MRI) may also be provided. This MRI consists of a PRRSV-negative serum sample that has been supplemented with a titrated PRRSV EU and PRRSV NA viral suspension.

BioSellal commercializes these reagents under the references listed below:

Table 2. Optional Reagents for Bio-T kit® PRRSV*

Reagent	Description	Provider	Cat. N°
PRRSV EU RNA	Quantified PRRSV EU RNA (6 x 10^4 copies/qRT-PCR)	BioSellal	cARNPRRSVEU-002
PRRSV NA RNA	Quantified PRRSV NA RNA (12 x 10^4 copies/qRT-PCR)	BioSellal	cARNPRRSVNA-002
PRRSV EU viral suspension	PRRSV EU viral suspension (10^3 TCID50/ml)	BioSellal	SV-PRRSV-EU-002
PRRSV NA viral suspension	PRRSV NA viral suspension ($10^{4.9}$ TCID50/ml)	BioSellal	SV-PRRSV-NA-002
MRI for serum sample	PRRSV EU/NA serum (100 x LD_{METHOD})	BioSellal	MRI-PRRSV-001

*These reagents are available only upon request, please contact BioSellal (contact@biosellal.com).

For Bio-T kit® PRRSV DIVA :

Synthetic RNA of PRRSV EU other than Suvaxyn® and of Suvaxyn® vaccine strain (titrated in number of copies/qRT-PCR) used by BioSellal for the validation of the kit, can be used to confirm performances of your thermal cycler(s). BioSellal sells these reagents under the following references:

Table 3. Optional reagents for Bio-T kit® PRRSV DIVA*

Reagent	Description	Provider	Cat. N°
RNA PRRSV EU other than Suvaxyn®	Quantified RNA of PRRSV EU other than Suvaxyn® (1.2x 10^5 copies/qRT-PCR)	BioSellal	cARNPRRSVEU-003
RNA Suvaxyn® vaccine strain	Quantified RNA of Suvaxyn® vaccine strain (1.2x 10^5 copies/qRT-PCR)	BioSellal	cARNPRRSVVAC001

*These reagents are available only upon request, please contact BioSellal (contact@biosellal.com).

For Bio-T kit® PCV3 and PCV2 & PCV3:

To confirm performances of your thermal cycler(s), for absolute quantification, or to constitute the reference material set at the interpretation threshold (MRSI), PCV2 or PCV3 DNA (quantified in copies/qPCR, cADN-PCV2-001 / cADN-PCV3-001) used by BioSellal in the validation file, are required. Ready to use MRSI may also be provided. These MRSI consist of a PCV2 and PCV3 negative blood sample that have been supplemented with a titrated DNA of PCV2 or PCV3 at a level of 10^6 GE / ml of blood, a level considered to be an indicator of high viremia for PCV2 and extrapolated to PCV3.

BioSellal commercializes these reagents under the references listed below:

Table 4. Optional Reagents for Bio-T kit® PCV3 and PCV2 & PCV3*

Reagent	Description	Provider	Cat. N°
PCV2 DNA	Quantified PCV2 DNA (1 x 10^6 copies/qPCR)	BioSellal	cADN-PCV2-001
PCV3 DNA	Quantified PCV3 DNA (1 x 10^6 copies/qPCR)	BioSellal	cADN-PCV3-001
PCV2 MRSI for blood sample	Blood positive for PCV2 at 10^6 GE / ml	BioSellal	MRSI-PCV2-001
PCV3 MRSI for blood sample	Blood positive for PCV3 at 10^6 GE / ml	BioSellal	MRSI-PCV3-001

*These reagents are available on request only, contact BioSellal (contact@biosellal.com).

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 Master Mix storage, qPCR plates preparation), "Nucleic acids (NA) Addition" (Nucleic Acid storage and addition of extracted Nucleic Acid and controls in the qPCR plate), "PCR" (final area containing the thermocycler(s)).
- Use dedicated equipment for each working area (gloves, lab coat, pipettes, vortex, ...).
- Use filter tips.
- Before use, thaw all components at room temperature.
- Vortex and spin briefly (mini-centrifuge) all reagents before use.
- It is recommended to not exceed 3 freeze-thawing cycles for samples, lysates, reagents and extracted nucleic acids. Depending on the use, we recommend to make aliquots using appropriate volume.
- Genomes of pathogens detected by the PIG line 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.
○ 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°C±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…).

VALIDATED EXTRACTION METHODS

GENERALITY

Recommendations for sampling, shipping and storage of samples

Real-time qPCR or qRT-PCR are powerful technique allowing the detection of few amounts of pathogen genome. Genome can be rapidly degraded depending on the pathogen nature (bacteria / parasites, enveloped viruses...), the genome nature (DNA / RNA) and the sample type (presence of DNase / RNase). Thus, BioSellal recommends the following instructions to guarantee an optimal diagnosis.

Sampling

- EDTA whole blood, serum
- Cell culture supernatant
- Oral Fluids
- Organs
- Individual test

To prevent cross-contamination between samples leading to false positive results, it is mandatory to use disposable materials for single use and to avoid direct contact between specimens.

Shipping

For circoviruses diagnosis:
It is recommended to ship soon as possible after sampling, under cover of positive cold.
For PRRSV diagnosis or in case of differential diagnosis for circoviruses and PRRSV on the same sample:
It is mandatory to ship immediately after sampling or by default to store it at ≤ -16°C. Shipment has to be done within 24h under cover of positive cold.

Storage after reception

For circoviruses diagnosis:
Recommended storage of samples at 5°C ± 3 for a maximum of 7 days and ≤ -16°C beyond.
For PRRSV diagnosis or in case of differential diagnosis for circoviruses and PRRSV on the same sample:
It is recommended to immediately analyze samples after receipt or freezing at ≤ -16 ° C for a few months and ≤ -65°C beyond 1 year.

Extraction kits

BioSellal proposes two extraction kits:

- BioExtract® Column Cat. N° BEC050 or BEC250 based on the use of silica columns, recommended for the extraction of 1 to 12-20 samples in parallel.
- BioExtract® SuperBall® Cat. N° BES384 based on the use of magnetic beads and the use of platform such as KingFisher™ Duo, mL, Flex or 96, recommended for the parallel extraction of 12 or more samples.

A simplified protocol for each method is proposed below. For more information, contact our technical support or www.biosellal.com.

Summary of Nucleic Acid Extraction Methods

Samples type	Concerned Bio-T kit®	Pre-treatment		Analysis: Individual or Pool	Extraction Method		Sample Volume µl	Elution Volume µl
		YES/ NO	Page		Name	Page		
Cell culture supernatant	PRRSV	NO		Individual analysis	BioExtract® Column	12	**100**	**60**
					BioExtract® SuperBall®	15		
Blood Serum	PRRSV PRRSV DIVA PCV3 PCV2 & PCV3	NO		Individual analysis or by pool up to 10	BioExtract® Column	12	**100**	**60**
					BioExtract® SuperBall®	15		
Oral Fluids	PRRSV PRRSV DIVA PCV3 PCV2 & PCV3	YES	10	Individual analysis	BioExtract® Column	12	**200**	**60**
					BioExtract® SuperBall®	15		
Organs	PRRSV PRRSV DIVA PCV3 PCV2 & PCV3	YES	10	Individual analysis	BioExtract® Column	12	**200**	**60**
					BioExtract® SuperBall®	15		

REQUIRED CONTROLS

Negative Control Sample (NCS):

For each extraction method, it is **compulsory** to include at least one NCS sample, in order to validate the absence of inter-contamination of the samples over the entire process. It is placed in the last position, after the samples to be extracted, and it is recommended to adapt the number of NCS to the level of risk of inter-contamination of the laboratory: for example, an NCS every 7-10 samples field.
For this control, the **sample volume of 100 µl or 200 µl** is replaced by **the same volume of PBS or water (RNase/DNase free)** and is treated in parallel with the field samples **throughout the extraction process**.

Positive Control Sample, MRI and if available MRSI

For the detection of PRRSV in all sample type or PCV2 and PCV3 in oral fluids or organs, a positive sentinel process control (MRI), consisting of a **low positive sample** (whole blood, serum, oral fluids, organs or cell culture supernatant) is extracted at the same time as the samples in one or more specimens (depending on the number of samples analysed). After qRT-PCR or qPCR, Ct values of this extraction indicator will be transferred and tracked over time on a control card. The fact of obtaining, after extraction and qRT-PCR/qPCR expected Ct values with this positive control validates the entire method.

For the detection of PCV2 and PCV3 in blood and serum, a reference material set at the interpretation threshold (MRSI), consisting of a **negative blood sample for PCV2 and PCV3** supplemented at **10^6 GE / ml** with a quantified PCV2 or PCV3 DNA, is extracted at the same time as the samples in one or more specimens (depending on the number of samples analysed). This level is considered as the limit between weak and high viremia for PCV2 and extrapolated to PCV3. After qPCR, Ct values of this extraction indicator will be transferred and tracked over time on a control card. The fact of obtaining, after extraction and qPCR expected Ct values with this positive control validates the entire method.
The MRSI load (10^6 GE / ml of blood) serve as a threshold to identify high viral load (> 10^6 GE / ml) and low viral load (<10^6 GE/ ml).

Note: **Exogenous IPC** IPC-A (PCV3, PCV2 & PCV3, tube with **pink** cap) **or** IPCRNA-A (PRRSV, PRRSV DIVA, tube with **purple** cap) supplied in Bio-T kit® PRRSV, PRRSV DIVA, PCV3, PCV2 & PCV3, should be used during extraction for each sample and extraction controls (NCS, MRI, MRSI). In case of differential diagnosis on the same sample, **both IPC-A** (tube with **pink** cap) **and IPCRNA-A** (tube with **purple** cap) supplied in Bio-T kit® PRRSV, PRRSV DIVA, PCV3, PCV2 & PCV3**, should be used.**

PRETREATMENT OF SAMPLES

Blood samples, serum or cell culture supernatants do not require pretreatment.
For the other samples, pretreatment protocols require the use of the ATL buffer (BioSellal Cat No. ATL19076).

For Organs :

Enzymatic lysis

1. Place **20 to 25 mg** of tissue (precision balance) into a sterile container (e.g.: Petri dish).
2. Proceed to a meticulous **dissection** with a sterile scalpel.
3. Place it into a **microtube** containing **1 to 2 glass beads** (2mm diameter) and **180 µl of ATL Buffer + 20 µl of Proteinase K*. Vortex for 30 seconds.**
4. Incubate for **15 minutes at 56°C** ± 1.5 (dry bath).
5. **Vortex for 15 seconds** and **centrifuge briefly**.

Pursue on **200 µl of pretreated sample** with the nucleic acid extraction and purification by following the BioExtract® Column (Cat. N° BEC050) or BioExtract® SuperBall® (Cat. N° BES384) protocols described below.

Mecanic lysis

1. Place **20 to 25 mg** of tissue (precision balance) into a sterile container (e.g.: Petri dish).
2. Place it into a microtube containing **1 to 2 glass beads** (2mm diameter) and **180 µl of ATL Buffer + 20 µl of Proteinase K***. **Vortex for 30 seconds.**
3. **Grind the organ.** Adapt your grinding conditions (speed and time) according to your grinding device and the type of organ to make the lysis condition optimal. For example, BioSellal recommends:
   - 2 minutes at 30 Hz (e.g.: Retsch™)
   - 2 x 30 secondes at 6 m/s (e.g.: FastPrep™)
   - 2 x 90 secondes at 6800 rpm (e.g.: Precellys™)
4. **Centrifuge briefly.**

Pursue on **200 µl of pretreated sample** with the nucleic acid extraction and purification by following the BioExtract® Column (Cat. N° BEC050) or BioExtract® SuperBall® (Cat. N° BES384) protocols described below.

For Oral Fluids :

1. **Conscientiously vortex the oral fluid** sample until total homogenization.
2. Into a 1.5 ml micro-centrifuge tube, add:
   - **300 µl oral fluid sample**,
   - and **300 µl ATL Buffer**.
3. Incubate for **5 minutes at 56°C** ± 1.5 (dry bath).
4. **Vortex for 15 seconds.**
5. Centrifuge at **10 000 x g for 2 minutes**.

Pursue on **200 µl of pretreated sample** with the nucleic acid extraction and purification by following the BioExtract® Column (Cat. N° BEC050) or BioExtract® SuperBall® (Cat. N° BES384) protocols described below.

* Proteinase K is supplied in BioExtract® kits

REALIZATION OF SAMPLES POOL FOR SAMPLE WITHOUT PRETREATMENT

For whole blood and serum

1. Proceed to the pool of samples up to 10 as following:
   a. Vortex each individual sample.
   b. Transfer **50 µl** of each sample in a tube.
      Note: for pool less than 3 samples, transfer 100 µl and proceed as described.
   c. Vortex to homogenize.
2. Pursue with the extraction and purification of the nucleic acids from 100 µl of pooled samples following the BioExtract® SuperBall® or BioExtract® Column described below.

NUCLEIC ACIDS EXTRACTION ON COLUMN

BioExtract® Column
Cat. N° BEC050 or BEC250

Refer to the handbook of the extraction kit for buffer reconstitution

1. Lysis and Adjustment of adsorption conditions

For blood, serum and cell supernatant:

1. Into a 1.5 ml tube, add:
   - **20 µl of Proteinase K**§¥
   - **100 µl of sample or PBS** (NCS)
   - **100 µl**¥ **of lysis solution LA-carrier + exogenous IPC°**, previously prepared according to Table 5
2. Vortex and incubate **15 min at 15–25°C**
3. Centrifuge briefly
4. Add **350 µl of LB buffer**.
5. Vortex and Centrifuge briefly

For oral fluids and organs:

1. Into a 1.5 ml tube, add:
   - **200 µl of pretreated sample or PBS** (NCS)
   - **100 µl of lysis solution LA-carrier + exogenous IPC°** previously prepared according to Table 5
   - **350 µl of LB buffer**.
2. Vortex and Centrifuge briefly

Table 5. Lysis solution LA-carrier + exogenous IPC°

Reagent	Number of samples 1	6*	12*	24*	30*
LA Buffer	100 µl	660 µl	1.32 ml	2.64 ml	3.3 ml
Carrier RNA (1 µg/µl)	1 µl	6.6 µl	13.2 µl	26.4 µl	33 µl
Exogenous IPC° (tube pink and/or purple cap)	5 µl	33 µl	66 µl	132 µl	165 µl

§ Proteinase K is supplied in BioExtract® kits

* In order to compensate for pipetting error, the prepared volume is 110% of the required volume

° Add exogenous IPC Cat N° IPC-A for Bio-T kit® PCV3 et PCV2 & PCV3 (tube pink cap) or exogenous IPC Cat N° IPCRNA-A for Bio-T kit® PRRSV and PRRSV-DIVA, (tube purple cap). For differential diagnosis of porcine cirvovirus and PRRSV on the same sample, add both exogenous IPC (IPC-A and IPCRNA-A). In this case, add 5µl of both IPC by sample and then distribute 120 µl of LA-carrier + exogenous IPC lysis solution in each tube of interest.

¥Note: Instead of dispensing Proteinase K in each tube just before adding the sample, it can be added to the LA-carrier lysis + exogenous IPC solution. In this case, it is necessary to ensure that Proteinase K does not remain in contact with the LA-carrier lysis + exogenous IPC solution for more than 10 minutes (extemporaneous addition, distribution in the tubes, execution of the protocol sequence).

The volume of Proteinase K to be added to the lysis solution follows the same 10% margin rule (e.g.: for 6 samples, 132 µl of Proteinase K must be added).

Then distribute 120 µl of LA-carrier + exogenous IPC+ Proteinase K lysis solution in each tube of interest. Excess solution volume cannot be retained.

2. Adsorption on silica membrane

1. **Carefully transfer the entire volume** on the **BioExtract® Mini Spin Column** (already placed on a 2 ml collection tube).
2. Centrifuge at **6 000 x g** for **1 minute**. Change the collection tube (Place the BioExtract® column in a new collection tube and discard the tube containing the filtrate).

3. Washes and Drying of the silica membrane

1. Add **600 µl of W1 buffer**.
2. Centrifuge at **6 000 x g** for **1 minute**. Change the collection tube.
3. Add **600 µl of W2 buffer.**
4. Centrifuge at **6 000 x g** for **1 minute**. Change the collection tube.
5. Centrifuge at **20 000 x g** for **2 minutes** (or **16 000 x g for 3 minutes**) to dry the membrane.

4. Elution of nucleic acids

1. Place the BioExtract® Mini Spin Column in a new clean tube of 1.5 ml (not provided), and discard the collection tube containing the filtrate.
2. Add gently **60 µl of EL buffer** (at room temperature) onto the center of the membrane.
3. **Incubate at room temperature** (15–25°C) for **1 minute**.
4. Centrifuge at **20 000 x g** for **1 minute** (or **16 000 x g for 2 minutes**).
5. Conserve the eluate into the 1.5 ml tube and discard the column.

5. Storage after extraction

The extracted nucleic acids can be stored at 5°C ± 3 if the qPCR or qRT-PCR is done within one hour following the extraction, otherwise it is recommended to store it at ≤ -16°C for 6 months or at ≤ -65°C for a better conservation.

Figure 1. Extraction with BioExtract® Column (Cat. N° BEC050 or BEC250)

Step	Procedure
1 **Lysis and Adjustment of adsorption conditions**	<u>For blood, serum and cell supernatant:</u> - **20 µl of Proteinase K¥** - **100 µl of sample** - **100 µl¥ of lysis solution LA-carrier+ exogenous IPC°** Vortex and incubate 15 min at 15–25°C Centrifuge briefly Add 350 µl of LB buffer. Vortex and Centrifuge briefly <u>For oral fluids and organs:</u> - **200 µl of pretreated sample** - **100 µl of lysis solution LA-carrier+ exogenous IPC°** - **350 µl of LB buffer.** Vortex and Centrifuge briefly
2 **Adsorption on the silica membrane**	Load the BioExtract® Mini Spin Column Carefully 6 000 x g 1 min
3 **Wash** **Drying the silica membrane**	1st Wash — 600 µl W1 — 6 000 x g 1min 2nd Wash — 600 µl W2 — 6 000 x g 1min - — - — 20 000 x g 2min *or 16 000 x g 3 min*
4 **Elution of nucleic acids**	**60 µl** of EL Buffer (RT) RT 1 min 20 000 x g 1min *or 16 000 x g 3 min*

° Add exogenous IPC Cat N° IPC-A for Bio-T kit® PCV3 et PCV2 & PCV3 (tube pink cap) or exogenous IPC Cat N° IPCRNA-A for Bio-T kit® PRRSV and PRRSV-DIVA, (tube purple cap). For differential diagnosis of porcine circovirus and PRRSV on the same sample, add both exogenous IPC (IPC-A and IPCRNA-A). In this case, add 5µl of both IPC by sample and then distribute 120 µl of LA-carrier + exogenous IPC lysis solution in each tube of interest.

NUCLEIC ACIDS EXTRACTION WITH MAGNETIC BEADS

BioExtract® SuperBall® Kit

Cat. N° BES384

For use on KingFisher™ Devices as Flex, 96, Duo or mL

Refer to the handbook of the extraction kit for solution reconstitution

1. Preparation of plates or strips

1. Prepare the consumables for the extraction:
   Flex or 96: 4 plates Deep-well and 2 microplates. Annotate according the element to add (see Table 7).
   Duo: 1 plate Deep-well and 1 elution strip.
   mL: 1 strip per sample. Get out the sliding worktable from the workstation and place the strips on it.

2. Add in the « Deep-well Lysate » plate (Flex or 96), in the raw A (Duo) or in position A of the strips (mL):
   - **20 µl of Proteinase K[§¥]**.
   - **Sample :**
     - **For blood, serum and cell supernatant : 100 µl**
     - **For oral fluids and organs: 200 µl of pretreated sample**
   - **500 µl[¥] of LAB-SMB-carrier lysis solution + exogenous IPC[o]** thoroughly vortexed for 30 seconds. See Table 6 :

Table 6. Lysis solution LAB-SMB-carrier + exogenous IPC[o]							
Reagent	**Number of samples***						
	1	**5**	**10**	**12**	**15**	**48**	**96**
LA Buffer	100 µl	550 µl	1.1 ml	1.32 ml	1.65 ml	5.28 ml	10.56 ml
LB Buffer	400 µl	2.2 ml	4.4 ml	5.28 ml	6.6 ml	21.12 ml	42.24 ml
SMB (SuperBall Magnetic Beads)‡	25 µl	137.5 µl	275 µl	330 µl	412.5 µl	1.32 ml	2.64 ml
Carrier RNA (1 µg/µl)	1 µl	5.5 µl	11 µl	13.2 µl	16.5 µl	52.8 µl	105.6 µl
Exogenous IPC[o] (tube pink and/or purple cap)	5 µl	27.5 µl	55 µl	66 µl	82.5 µl	264 µl	528 µl

[§] Proteinase K is supplied in BioExtract® kits
* In order to ensure the pipetting volume, the prepared volume includes 10% additional volume relative to the volume required. The excess buffer volume may be retained for use within 8 days; beyond this period, it must be discarded.
‡ **Vortex vigorously for 3 minutes before first use or 1 minute for the following uses.**

° Add exogenous IPC Cat N° IPC-A for Bio-T kit® PCV3 et PCV2 & PCV3 (tube pink cap) or exogenous IPC Cat N° IPCRNA-A for Bio-T kit® PRRSV and PRRSV-DIVA, (tube purple cap). For differential diagnosis of porcine cirvovirus and PRRSV on the same sample, add both exogenous IPC (IPC-A and IPCRNA-A). In this case, add 5µl of both IPC by sample and then distribute 500 µl of LAB-SMB-carrier +exogenous IPC lysis solution in each tube of interest.

¥Note: Instead of dispensing Proteinase K into each well just before adding the sample, it can be added to the LAB-SMB-carrier lysis solution. In this case, it is necessary to ensure that Proteinase K does not remain in contact with the LAB-SMB-carrier lysis solution for more than 10 minutes (extemporaneous addition, distribution in the wells, launch of the program). The volume of Proteinase K to be added to the lysis solution follows the same rule of 10% margin (eg: for 5 samples, 110 µl of Proteinase K will have to be added).
Then distribute 520 µl of LAB-SMB-carrier + exogenous IPC + Proteinase K lysis solution in each well of interest. Excess solution volume cannot be retained.

3. Fill deepwell or plates according to Table 7 below:

Table 7. Volume of reagents and Configuration of automates KingFisher™ Flex, 96, Duo et mL

Position			Element to add	Volume per well (µl)
Flex	**Duo***	**mL**		
Deep-well Lysat	Line A	Position A	Lysate†	620-720†
Deep-well Wash 1	Line E	Position B	Buffer W1	700
Deep-well Wash 2	Line F	Position C	Buffer W2	700
Deep-well Wash 3	Line G	Position D	Ethanol (96–100%)	750
Elution Microplate	Elution strip	Position E	Buffer EL	60
Rod cover (Large 96-Rod Cover)	Line B	*To place manually*	Rod cover	—

* Row C, D and H are empty
† Includes 20 µl Proteinase K, sample and 500 µl LAB-SMB-carrier+exogenous IPC Lysis solution.

2.Launch of the KingFisher™

1. Select the program « **BioExtract_KF_Flex** », « **BioExtract_KF_96** », « **BioExtract_KF_Duo** » or « **BioExtract_KF_mL** » and load the plates into the workstation. Start the run.
2. At the end of the extraction program, keep the elution plate containing the nucleic acids extracts and discard the other plates.

3.Storage after extraction

The extracted nucleic acids can be stored at 5°C ± 3 if the qPCR or qRT-PCR is done within one hour following the extraction, otherwise it is recommended to store it at ≤ -16°C for 6 months or at ≤ -65°C for a better conservation.

Figure 3. Extraction with BioExtract® SuperBall® kit (Cat. N° BES384)

	KingFisher™ Flex or 96	KingFisher™ Duo	KingFisher™ mL	Element to add
1 Plate or Strip Preparation	Deep-well Lysate	Row A	Position A	**Lysate :** 20 µl Proteinase K; For blood, serum and cell supernatant: 100 µl of sample; For oral fluids and organs: 200 µl of pretreated of sample; 500 µl LAB-SMB-carrier Lysis Solution+ Exogenous IPC°
	Deep-well Wash 1	Row E	Position B	700 µl W1 Buffer
	Deep-well Wash 2	Row F	Position C	700 µl W2 Buffer
	Deep-well Wash 3	Row G	Position D	750 µl Ethanol (96-100%)
	Elution microplate	Elution strip	Position E	60 µl EL Buffer
	Rod cover microplate	Row B (Rows C, D and H are empty)	*Rod cover placed manually*	Rod Cover
2 KingFisher™	• Switch on the KingFisherTM Flex, 96 Duo or mL. • Slide open the front door of the protective cover. • Select the corresponding BioExtract® SuperBall® program. • Press START and follow the messages to load the different slots of the worktable.			

° Add exogenous IPC Cat N° IPC-A for Bio-T kit® PCV3 et PCV2 & PCV3 (tube pink cap) or exogenous IPC Cat N° IPCRNA-A for Bio-T kit® PRRSV and PRRSV-DIVA, (tube purple cap). For differential diagnosis of porcine cirvovirus and PRRSV on the same sample, add both exogenous IPC (IPC-A and IPCRNA-A). In this case, add 5µl of both IPC by sample and then distribute 500 µl of LAB-SMB-carrier +exogenous IPC lysis solution in each tube of interest.

To obtain the BioExtract® KingFisher™ program, please contact our technical support (tech@biosellal.com).

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