HANDBOOK

BioExtract® SuperBall®

Cat. N° BES384 and BES384WO

ALL SPECIES

Extraction and Purification of Total Nucleic Acids by magnetic beads using devices equivalent to KingFisher™ Flex, 96, Duo, mL
For pathogens detection from animal samples or environment:

- **viral RNA/DNA**
- **bacterial DNA**
- **parasite DNA**
- **genomic RNA/DNA**

Veterinary use only

DOCUMENTS MANAGEMENT

The BioExtract® SuperBall® handbook describes the composition of the kit, the different step for the buffer reconstitution and the general principle of kit use. The last version in use is indicated on the certificate of analysis (CA) provided with the BioExtract® SuperBall® kit.

Besides this handbook, each Bio-T kit® have their own technical handbook:

- The extraction handbook, displaying BioSellal's validated/or recommended extraction protocols for each type of sample.
- The qPCR handbook, presenting the instruction information to perform the qPCR.

Please refer to the extraction handbook of your specific Bio-T kit® to obtain the detailed protocol for each sample type.

REVISION MANAGEMENT

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

Revision management			
Type of modification Highlighting colour	**Minor Modifications**	**Main Modifications 1**	**Main Modifications 2**
Impact on the 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	Change of reference of non-critical reagent	Changing the composition of a critical reagent
	Addition of new sample type for extraction	Change in packaging volume of a critical reagent	Modification of validated extraction protocol
	Addition of information giving more details or alternative protocol		

PRESENTATION

Kit contents and storage

BioExtract® SuperBall® Kit (Cat No. BES384 and BES384WO) includes reagents in sufficient volume to achieve 384 independent extraction-purifications.
In addition, BioExtract® SuperBall® Kit (Cat No. BES384) includes consumables to perform four complete runs on KingFisher Flex or 96 (combs, deep-well bottom U for washing steps and elution plates). For other types of devices, use the specific plastics recommended by the supplier.
BioExtract® SuperBall® kit (Cat. N° BES384 and BES384WO) should be stored at room temperature (15-25 °C) until the expiration date stated on the label and indicated on the certificate of analysis (CA).

Table 1. Kit contents and Storage conditions

Description	Volume and units number		Storage	Reconstitution requires?
	Cat N°BES384	Cat N°BES384WO		
LA Buffer	2 x 30 ml	2 x 30 ml	15°C - 25°C	NO
LB Buffer (concentrated)	2 x 60 ml	2 x 60 ml	15°C - 25°C	**YES**
Proteinase K	2 x 6 ml	2 x 6 ml	15°C - 25°C, protected from light	NO
RNA Carrier (poly A)	2 x 310 µg	2 x 310 µg	Lyophilized: 15°C - 25°C Reconstituted: in aliquots at ≤- 16°C	**YES**
SuperBall® Magnetic Beads (SMB)	13 ml	13 ml	15°C - 25°C	NO
W1 Buffer (concentrated)	151 ml	151 ml	15°C - 25°C	**YES**
W2 Buffer (concentrated)	108 ml	108 ml	15°C - 25°C	**YES**
EL Buffer	125 ml	125 ml	15°C - 25°C	NO
Large 96-Rod Cover	4	-	-	-
Deep-well	20	-	-	-
Elution Microplate	4	-	-	-
Handbook	1	1	-	-

Reagents not supplied in the kit

Table 2. Reagents not supplied in the kit

Reagent	Description	Provider*	Cat. N°
Ethanol	Absolute Ethanol molecular biology grade *or equivalent*	VWR	20821.296
Isopropanol	Propanol molecular biology grade *or equivalent*	VWR	437423R
Concentrated solution for EL Coloration	40X Yellow Colored buffer for easy tracking during pipetting	BioSellal	TPCOL

* Indicative list of providers

General precautions

⚠ **CAUTION: DO NOT add bleach or acidic solutions directly in the liquid waste containing LA, LB and W1 buffers. Indeed, they contain a chaotropic salt that can form a highly reactive component in presence of bleach or acid solution.**

- Wear appropriate personal protective equipment adapted to the pathogenic risk (lab coat, disposable gloves frequently changed).
- Use filter tips.
- During the extraction it is mandatory to handle under the MSC untill the end of sample lysis, because of the zoonotic risk associated with the manipulated sample types and the pathogenic agents presence.

Important points before to start

- Check that the BioExtract® programs «BioExtract_KF_Flex», « BioExtract_KF_96», «BioExtract_KF_Duo» or «BioExtract_KF_mL» are installed on your KingFisher™ device.
  For each Bio-T kit®, the classical extraction program (38 minutes) has been validated. For some Bio-T kits® and/or some sample type, a short program (19 minutes) has also been validated. Please contact the technical support for more information's.

- It is mandatory to include a « negative control » (NCS) to verify the absence of cross contamination between samples during the extraction. The sample is replaced by water (RNase/DNase free) and will be processed in parallel of the samples.

- Following the information given in Table 3 below: Reconstitute the carrier RNA and prepare LB, W1 and W2 buffers or check that the buffers have been prepared according to the instructions below.

Table 3. Reagents Preparation

Reagent	Preparation
RNA carrier *	Add 310 µl Buffer EL to lyophilized 'carrier RNA'
Buffer LB†	Add 40 ml Isopropanol (100%) to Buffer LB
Buffer W1†	Add 200 ml Ethanol (96-100%) to Buffer W1
Buffer W2†	Add 252 ml Ethanol (96-100%) to Buffer W2

* The 'RNA carrier' dissolved in Buffer EL should be frozen in aliquots at -20 ° C. The aliquots of 'carrier RNA' must not be thawed and frozen more than 3 times.
† All reconstituted buffers and reagents are stable until the expiration date printed on the box of the kit at room temperature (15-25 ° C) without affecting the performance of the kit.

- For KingFisher™ Flex or 96, the 96-rod covers are supplied as bags of 2. If opening a new bag, store the second 96-rod cover on a Deep-well. Care should be taken to not bend the 96-rod covers.

PROCEDURE

1. Prepare the extraction consumables – Annotate it depending on the element to add (see Table 5):

- KingFisher™ Flex or 96:
  - 4 Deep-wells (V Bottom for lysis step and U or V Bottom for washing steps).
  - 1 elution Microplate
  - 1 Rod-cover placed in a reusable elution Microplate

- KingFisher™ Duo:
  - 1 Deep-well (Warning: for the Duo, the U Bottom Deep-wells provided in the kit are not compatible)
  - 1 Elution strip (not provided in the kit).

- KingFisher™ mL:
  - 1 strip per sample (not provided in the kit). Get out the sliding worktable from the workstation and place the strips on it.

2. Prepare the LAB-SMB-carrier Lysis Solution (See Table 4 below).
Make sure that the SMB solution is totally suspended: vortex for 3 minutes before first use, or 1 minute for the following uses.
It is possible to prepare an excess volume of LAB-SMB-carrier Lysis Solution and keep it at room temperature for a use within 8 days.
Exogenous IPC addition is mandatory, optional or not required depending on the Bio-T kit®. Refer to the extraction handbook of your specific Bio-T kit®.

Table 4. Preparation of lyse LAB-SMB-carrier

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
Buffer LB	400 µl	2.2 ml	4.4 ml	5.28 ml	6.6 ml	21.12 ml	42.24 ml
SMB (Super®Ball® Magnetic Beads) ‡	25 µl	137.5 µl	275 µl	330 µl	412.5 µl	1.32 ml	2.64 ml
RNA Carrier (1 µg/µl)	1 µl	5.5 µl	11 µl	13.2 µl	16.5 µl	52.8 µl	105.6 µl
Exogenous IPC†	5 µl	27.5 µl	55 µl	66 µl	82.5 µl	264 µl	528 µl

* To guarantee the pipetted volume, the prepared volume contains a supplementary volume of 10%. The exceeding volume of lysis solution can be stored for maximum 8 days, beyond this duration, the solution must be discarded.
‡ Thoroughly vortex for 3 minutes before first use and for 1 minutes for the following uses.
† IPC Volume recommended in Bio-T kits® (kits of detection by qPCR BioSellal). Refer to the extraction handbook of each Bio-T kit® or contact BioSellal Technical Service (tech@biosellal.com).

3. Add in the « Deep-well lysate » plate for KingFisher™ (Flex or 96), in the Row A for KingFisher™ (Duo) or in Position A for KingFisher™ (mL):

- Add **20 µl of Proteinase K**[¥].
- Add **100 to 200 µl of sample**, depending on the matrix and protocol recommended by BioSellal.
- Vortex strongly LAB-SMB-carrier solution for 30 seconds then add **500µl Lysis Solution LAB-SMB-carrier** to each sample.

[¥]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 (± exogenous IPC). In this case, it is necessary to ensure that Proteinase K does not remain in contact with the LAB-SMB-carrier (± exogenous IPC) 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 (e.g.: for 5 samples, 110 µl of Proteinase K will have to be added).
Then distribute 520 µl of LAB-SMB-carrier + Proteinase K (± exogenous IPC) lysis solution in each well of interest. Excess solution volume cannot be retained.

4. Prefill deep-well plates and microplates according to Table 5 below:
It is possible to prepare plates of reagents of Buffers W1, W2, W3 and EL in advance for a use within 8 days. Seal the filled plates to prevent evaporation.

Table 5. KingFisher™ Flex, Duo and mL Configuration and Reagent volume

Position on the strip or on the plate			Element to add	Volume per well (µl)
Flex	Duo*	mL		
Deep-well Lysate	Row A	Position A	Lysate†	720
Deep-well Wash 1	Row E	Position B	Buffer W1	700
Deep-well Wash 2	Row F	Position C	Buffer W2	700
Deep-well Wash 3	Row G	Position D	Ethanol (96–100%)	750
Elution Microplate	Elution strip	Position E	Buffer EL	50-100 ‡
Rod Cover Microplate (Large 96-Rod Cover)	Row B	*Placed manually*	Rod cover §	—

* Rows C, D and H are empty.
† Includes 20 µl Proteinase K, 200 µl Sample and 500 µl LAB-SMB-carrier Lysis Solution.
‡ For the elution volume, refer to the manual of each Bio-T kit® or contact BioSellal Technical Service (tech@biosellal.com).

Note:
A yellow concentrated solution could be added to the EL buffer to facilitate the tracking of nucleic acid addition in the blue Master Mix. The mixed solution becomes green.
This yellow solution is provided at 40x concentrate and should be used at 1X concentration in EL buffer. For example, add 1 ml of yellow concentrated solution (TPCOL) to an aliquot of 39 mL of EL buffer. Vortex the obtained solution before the adding in the elution microplate.

5. Switch on the KingFisher™ Flex, Duo or mL.

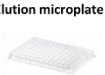
6. Select the program « BioExtract_KF_Flex », « BioExtract_KF_Duo » or « BioExtract_KF_mL » using the arrow keys.

7. Press START and follow the messages to load the plates into the workstation.

Depending on the Bio-T kit®, BioSellal has validated a classical extraction run lasting 38 minutes and a short extraction program lasting 19 minutes.

At the end of the program, recover the eluents from the KingFisher ™ Duo or mL or retrieve the elution plate from the KingFisher™ Flex or 96 devices. Refer to the handbook of the Bio-T kit® for the procedures for use and storage of nucleic acids extracts.

SIMPLIFIED PROTOCOL

	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 of Proteinase K 100-200 µl of pretreated 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 	**50-200 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 KingFisher™ 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.			

*For sample volume and elution volume, please refer to the specific extraction handbook of each Bio-T kit® or contact BioSellal Technical Service (tech@biosellal.com).

To get the KingFisher ™ program corresponding to the KingFisher™ system you are using (Flex, 96, Duo or mL), please contact our technical support (tech@biosellal.com).

www.biosellal.com

Technical Support

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Contact and Information

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