

HANDBOOK

BioExtract® Premium Mag

Cat. N° BEPM96 – 96 extractions
Cat. N° BEPM1K – 1 000 extractions
Cat. N° BEPM2K – 2 000 extractions
Cat. N° BEPM5K – 5 000 extractions

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 all species samples or environment:**

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

Research Use Only

DOCUMENTS MANAGEMENT

The BioExtract® Premium Mag handbook describes the composition of the kit, the different steps 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® Premium Mag kit.

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

- The extraction handbook, displaying BioSellal's validated / 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 (pre-treatment, volume of sample, elution volume).

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

For BEPM96:

BioExtract® Premium Mag Kit (Cat No. BEPM96) includes reagents in sufficient volume to achieve 96 independent extraction-purifications.

BioExtract® Premium Mag kit 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). **Only proteinase K and Poly-A-RNA tubes have to be stored at ≤-16°C.**

Table 1. Kit contents and Storage conditions for BEPM96

Package	Description	Volume	Storage	Reconstitution requires?
Parcel sent at room temperature	Lysis Buffer	20 ml	15°C - 25°C	NO
	Binding Buffer	40 ml	15°C - 25°C	NO
	Wash Buffer I	80 ml	15°C - 25°C	NO
	Wash Buffer II	80 ml	15°C - 25°C	NO
	Wash Buffer III	80 ml	15°C - 25°C	NO
	Elution Buffer	20 ml	15°C - 25°C	NO
	Premium Beads	2 ml	15°C - 25°C	NO
Package received under dry ice	Proteinase K	1 ml	≤- 16°C	NO
	Poly-A-RNA	120µl	≤- 16°C	NO

If precipitation occurs in the buffer, heat them at 25-37 ° C to dissolve the precipitate before use.

For BEPM1K, BEPM2K and BEPM5K:

BioExtract® Premium Mag Kit (Cat No. BEPM1K, BEPM2K and BEPM5K) includes reagents in sufficient volume to achieve 1 000, 2 000 and 5 000 independent extraction-purifications respectively.

BioExtract® Premium Mag kit 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). **Only the lyophilized proteinase K and Poly-A-RNA tubes have to be stored at 5°C ± 3 until the resuspension of the vial.**

Table 2. Kit contents and Storage conditions for BEPM1K, BEPM2K and BEPM5K

Description	Volume			Storage	Reconstitution requires?
	BEPM1K	BEPM2K	BEPM5K		
Lysis Buffer	200 ml	2x 200 ml	1000 ml	15°C - 25°C	NO
Binding Buffer	400 ml	2x 400 ml	2 x 1000 ml	15°C - 25°C	NO
Wash Buffer I	800 ml	2x 800 ml	4 x 1000 ml	15°C - 25°C	NO
Wash Buffer II	800 ml	2x 800 ml	4 x 1000 ml	15°C - 25°C	NO
Wash Buffer III	800 ml	2x 800 ml	4 x 1000 ml	15°C - 25°C	NO
Elution Buffer	200 ml	2x 200 ml	1000 ml	15°C - 25°C	NO
Proteinase K	1 x 200 mg (for 1x10 ml of reconstituted solution)	2 x 200 mg (for 2x10 ml of reconstituted solution)	5x 200 mg (for 5x10 ml of reconstituted solution)	Lyophilized : 5°C ± 3 Reconstituted : aliquote part at ≤ -16°C	YES
Poly-A-RNA	3 mg	2x 3 mg	15 mg	Lyophilized : 15°C -25°C Reconstituted : aliquote part at ≤ -16°C	YES
Poly-A-RNA Buffer	5 ml	2x 5 ml	20 ml	15°C - 25°C	NO
Lysis Buffer	200 ml	2x 200 ml	1000 ml	15°C - 25°C	NO

If precipitation occurs in the buffer, heat them at 25-37 °C to dissolve the precipitate before use.

Following the information given in Table 3 below: Reconstitute the Proteinase K and Poly-A-RNA or check that the buffers have been prepared according to the instructions below.

Table 3. Reagents Preparation for BEPM1K, BEPM2K and BEPM5K

Reagent	Preparation
Proteinase K	Add 10 ml of water in each vials of proteinase K (RNase / DNase free), vortex and store the aliquote part at ≤ -16 °C.
Poly-A-RNA	BEPM1K and BEPM2K Add 1.2 ml of Poly-A-RNA Buffer per tube , vortex and store the aliquote part at ≤ -16 °C.
	BEPM5K Add 6 ml of Poly-A-RNA Buffer , vortex and store the aliquote part at ≤ -16 °C.

Consumable for KingFisher™

Table 4. Consumable for KingFisher™			
Reagent	Description	Provider	Cat. N°
Deepwell 2ml	60 pieces/ box	BioSella	KF96DW-002
Elution plate	60 pieces/ box	BioSella	KF96MP-001
Rod cover	60 pieces/ box	BioSella	KF96RC-003

General precautions

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

- ⚠ **Some sample may contain pathogens with risks for animal and/or environment and/or Human. Refer to your local regulations for handling depending on pathogens and/or samples.**

- 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 until the end of sample lysis, because of the zoonotic risk associated with the manipulated sample types and the pathogenic agents presence.
- Infectious potential of liquid waste left over after using the BioExtract® Premium Mag Kit was not tested. Even though contamination of waste with residual infectious material is unlikely, it cannot be excluded completely. Therefore, liquid waste should be handled as being potentially infectious, and discarded according to local safety regulations

Important points before starting.

- Check that the program « Premium-Mag-KF » and/or « Premium-Mag-Fast-KF » is installed on your KingFisher™ device.

- 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.

PROCEDURE

1. Preparation of the plates or strips

1. Prepare the consumable for the extraction series (see Table 7):

- **KingFisher™ Flex or 96:**
 - 4 Deep-wells
 - 1 elution Microplate
 - 1 Rod-cover placed in elution Microplate
- **KingFisher™ Duo:**
 - 1 Deep-well
 - 1 Elution strip
- **KingFisher™ mL:**
 - 1 strip per sample. Get out the sliding worktable from the workstation and place the strips on it.

2. Add at the « Deep-well Sample» plate (Flex or 96), wells of the line A (Duo) or wells in the A position of the strap (mL), add:

- Add **200 µl of sample**, if the volume is lower, make up with 1X PBS.
- Add **630 µl of lysis Solution ± exogenous IPC**, previously vortexed. (See Table 5 or Table 6 below).

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®.

Make sure that the Premium Beads is totally suspended: vortex for 3 minutes before first use, or 1 minute for the following uses.

Without exogenous IPC :

Table 5. Preparation of lysis Solution							
Reagent	Number of samples*						
	1	5	10	12	15	48	96
Lysis Buffer	200 µl	1.1 ml	2.2 ml	2.64 ml	3.3 ml	10.56 ml	21.12 ml
Reconstituted Poly-A-RNA	1 µl	5.5 µl	11 µl	13.2 µl	16.5 µl	52.8 µl	105.6 µl
Reconstituted Proteinase K	10 µl	55 µl	110 µl	132 µl	165 µl	528 µl	1.06 ml
Premium Beads	20 µl	110 µl	220 µl	264 µl	330 µl	1056 µl	2.12 ml
Binding Buffer	400 µl	2.2 ml	4.4 ml	5.28 ml	6.6 ml	21.12 ml	42.24 ml

* To guarantee the pipetted volume, the prepared volume contains a supplementary volume of 10%.

With exogenous IPC :

Table 6. Preparation of lysis Solution+ exogenous IPC

Reagent	Number of samples*						
	1	5	10	12	15	48	96
Lysis Buffer	200 µl	1.1 ml	2.2 ml	2.64 ml	3.3 ml	10.56 ml	21.12 ml
Reconstituted Poly-A-RNA	1 µl	5.5 µl	11 µl	13.2 µl	16.5 µl	52.8 µl	105.6 µl
Reconstituted Proteinase K	10 µl	55 µl	110 µl	132 µl	165 µl	528 µl	1.06 ml
Premium Beads	20 µl	110 µl	220 µl	264 µl	330 µl	1056 µl	2.12 ml
Binding Buffer	400 µl	2.2 ml	4.4 ml	5.28 ml	6.6 ml	21.12 ml	42.24 ml
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%.

† IPC volume recommended in Bio-T kits® (kits of detection by qPCR BioSella). Refer to the extraction handbook of each Bio-T kit® or contact BioSella Technical Service (tech@biosella.com).

- Distribute the reagents, according to Table 6 below:

Table 7. Reagent volume and Device Configuration KingFisher™ Flex, 96, Duo and mL

Position on the strip or on the plate			Element to add	Volume per well (µl)
Flex	Duo*	mL		
Deep-well Sample plate	Row A	Position A	Sample + Lysis solution†	830†
Deep-well Wash 1	Row E	Position B	Wash Buffer I	800
Deep-well Wash 2	Row F	Position C	Wash Buffer II	800
Deep-well Wash 3	Row G	Position D	Wash Buffer III	800
Elution Microplate	Elution strip	Position E	Elution Buffer	60-200‡
Rod Cover Microplate (Large 96-Rod Cover)	Row B	<i>Placed manually</i>	Rod cover	—

* Rows C, D and H are empty.

† Include 200 µl of sample + 630 µl of lysis solution ±exogenous IPC

‡ Elution volumes depend on the Bio-T kit® concerned. If necessary, refer to the instructions for each detection kit for more information or contact the BioSella Technical Service (tech@biosella.com). In the absence of indications, BioSella recommends an elution volume of 100 µl

2. Launch of the KingFisher™

- Place the plates or strips into the device « Premium-Mag-KF » or « Premium-Mag-Fast-KF » program
- Press START and follow the messages to load the plates into the workstation. Make sure that all plates are inserted in the same orientation (especially when using partially filled plates).

‡ The 24-minute short program (Premium-Mag-Fast-KF) can be used for special applications. Please refer to the Bio-T kit® instruction manuals for the compatibility of this program.

3. Storage after the elution

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 Sample plate 	Row A 	Position A 	<p style="text-align: center;">Lysate : 200 µl of sample (or sample completed up to 200µl with 1X PBS) + 630 µl of Lysis solution ± Exogenous IPC</p> <p style="text-align: center;">800 µl Wash Buffer I</p> <p style="text-align: center;">800 µl Wash Buffer II</p> <p style="text-align: center;">800 µl Wash Buffer III</p> <p style="text-align: center;">60-200 µl of Elution Buffer</p> <p style="text-align: center;">Rod Cover</p>
	Deep-well Wash 1 	Row E 	Position B 	
	Deep-well Wash 2 	Row F 	Position C 	
	Deep-well Wash 3 	Row G 	Position D 	
	Elution microplate 	Elution strip 	Position E 	
	Rod cover microplate 	Row B  (Rows C, D and H are empty)	<i>Rod cover placed manually</i>	

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- KingFisher™**
- Switch on the KingFisher™ Flex, 96 Duo or mL.
 - Slide open the front door of the protective cover.
 - Select the « Premium-Mag-KF » or « Premium-Mag-Fast-KF » program.
 - Press START and follow the messages to load the different slots of the worktable.

The 24-minute short program (Premium-Mag-Fast-KF) can be used for special applications. To get the KingFisher™ program corresponding to the KingFisher™ system you use (Flex, 96, Duo or mL), please contact our technical support (tech@biosellal.com).



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