HANDBOOK

BioExtract® Column

Cat. N° BEC050 / BEC250

Extraction and Purification of Total Nucleic Acids Kit
(viral RNA/DNA; bacterial DNA; parasite DNA; genomic RNA/DNA)
by individual silica column
for the detection of pathogens
from samples of animal origin or their environment

Veterinary use only

DOCUMENTS MANAGEMENT

Bio-T kits® have their own technical manuals detailing for each type of sampling the methods of extraction validated by BioSellal and the different stages of preparation of q (RT) -PCR. The latest valid versions of these documents can be found in the Certificate of Analysis (CA) supplied with the Bio-T kits®.

In addition to these manuals, the general manual for use of the BioExtract® Column Kits describes the composition of the kit, the preliminary steps for the reconstitution of certain reagents as well as the general and common principle of use of the kit.

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

Reagents and consumables contained in the kit

The BioExtract® Column Kits (Cat No. BEC50 or BEC250) include reagents in sufficient volume to achieve 50 or 250 independent extraction-purifications.
BioExtract® Column (050) (Cat. N° BEC050) or (250) (Cat. N° BEC250) can be stored at room temperature (15-25 °C) until the expiration date stated on the label.

Table 1. Kit contents and Storage conditions

BioExtract® Column Kit Cat. N°/Number of preps	(50) BEC050/50	(250) BEC250/250	Storage conditions
BioExtract® Mini Spin Column	50	250	-
Collection tubes	200	1 000	-
Buffer LA*	6 ml	30 ml	15°C at 25°C
Buffer LB*† (concentrated)	12 ml	60 ml	15°C at 25°C
Proteinase K	1.25 ml	6 ml	15°C at 25°C
'Carrier RNA' (poly A)	310 µg	310 µg	Lyophilized : 15°C at 25°C Reconstituted : aliquoted at <-16°C
Buffer W1*‡ (concentrated)	19 ml	98 ml	15°C at 25°C
Buffer W2‡ (concentrated)	17 ml	81 ml	15°C at 25°C
Buffer EL§	20 ml	2 x 20 ml	15°C at 25°C
Protocol	1	1	-

* CAUTION : Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach.
† Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.
‡ Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.
§ CAUTION : Contains sodium azide as a preservative.

Reagents not supplied in the kit

Table 2. Reagents not supplied in the kit

Reagent	Description	Supplier*	Cat. N°
Tube 1.5 ml	microtube 1,5 ml pp safelock 3815 eppendorf	Dutscher	033290
Ethanol	Absolute Ethanol AnalaR NORMAPUR for analyses *or equivalent*	VWR	20821.296
Isopropanol	Propanol molecular biology grade *or equivalent*	VWR	437423R

* Indicative list of suppliers.

General precautions

- Wear the appropriate Individual Protection Equipment adapted to the pathogenic risk associated with the samples handled (lab coat, disposable gloves frequently changed,).
- ⚠ CAUTION: DO NOT add bleach or acidic solutions directly in the waste.
- Buffers LA, LB and W1 contain a chaotropic salt that can form a highly reactive component in presence of bleach.
- Use filter tips.
- Until the end of the sample lysis step, it is recommended to work under a PSM.

Important points before to start

- 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 4 below: Reconstitute the 'carrier RNA' and prepare Buffers LB, W1 and W2 or check that the Buffers have been prepared according to the instructions.

Table 3. Reagents Preparation

Reagent	Preparation	
	BEC050	**BEC250**
Carrier RNA*	Add 310 µl Buffer EL to lyophilized 'carrier RNA'	
Buffer LB†	Add 8 ml Isopropanol (100%) to Buffer LB	Add 40 ml Isopropanol (100%) to Buffer LB
Buffer W1†	Add 25 ml Ethanol (96-100%) to Buffer W1	Add 130 ml Ethanol (96-100%) to Buffer W1
Buffer W2†	Add 40 ml Ethanol (96-100%) to Buffer W2	Add 190 ml Ethanol (96-100%) to Buffer W2

* The 'carrier RNA' 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.

PROCEDURE

1. Lysis and Adjustment of adsorption conditions

Into a 1.5 ml micro-centrifuge tube (not provided):

- Add **20 µl Proteinase K**.
- Add **100 to 200 µl of vortexed sample**.
- Add **100 µl LA-carrier Lysis Solution** prepared according to Table 4 below.

Table 4. LA-carrier Lysis Solution

Reagent	Number of samples				
	1	**6***	**12***	**24***	**30***
Buffer LA	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 † (provided in Bio-T® kits)	5 µl	33 µl	66 µl	132 µl	165 µl

* In order to guarantee the pipetted volume, the prepared volume contains a supplementary volume of 10%.
† IPC volume advocated in Bio-T® kits (BioSellal detection kits). If necessary, refer to the instructions of each kit of detection or contact BioSellal Technical Support (tech@biosellal.com).

- Vortex and **incubate 15 min at room temperature** and centrifuge briefly.
- Add **350 µl Buffer LB**.
- Vortex and centrifuge briefly.

2. Adsorption on the silica membrane

- Carefully transfer the entire volume (570 to 670 µl) on the BioExtract® Mini Spin Column (placed into a clean 2 ml collection tube).
- Centrifuge at **6 000 x g for 1 min**.
- Change the collection tube (Place the BioExtract® Mini Spin Column into a clean collection tube and discard the collection tube containing the filtrate).

3. Washes and Drying of the silica membrane

- Add **600 µl Buffer W1**.
- Centrifuge at **6 000 x g for 1 min**. Change the collection tube.
- Add **600 µl Buffer W2**.
- Centrifuge at **6 000 x g for 1 min**. Change the collection tube.

- Centrifuge at **20 000 x g for 2 minutes** (or at 16 000 x g for 3 minutes) to dry the membrane.

4. Elution of Nucleic Acids

- Place the BioExtract® Mini Spin Column into a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate.
- Add gently **50-100 µl Buffer EL** (at room temperature) onto the center of the membrane.
- **Incubate at room temperature (15-25 ° C) for 1 min**.
- Centrifuge at **20 000 x g for 1 min** (or at 16 000xg for 2 minutes).

Transfer the eluate into a labelled tube or conserve the eluate into the 1.5 ml tube and discard the column.

The extracted RNA can be stored at 4°C if qRT-PCR is done within one hour following the extraction, otherwise it is recommended to store it at <-20°C for 6 months or at <-70°C for a better conservation.

SIMPLIFIED PROTOCOL

1 **Lysis and Adjustment of adsorption conditions**		**20 µl Proteinase K** **100 to 200 µl Sample** **100 µl of LA-carrier lysis solution** **(For 1 : 100 µl Buffer LA / 1 µl carrier RNA** **± 5 µl exogenous IPC)** **Room temperature (RT)** **15 min** **350 µl of Buffer LB**
2 **Adsorption on the silica membrane**		**Load the column BioExtract® Mini Spin Column carefully** **6 000 x g** **1 min**
3 **Washings** **Drying the silica membrane**		**1st Wash** — **600 µl W1** — **6 000 x g, 1 min** **2nd Wash** — **600 µl W2** — **6 000 x g, 1 min** **-** — **-** — **20 000 x g, 2 min (or 16 000 x g 3min)**
4 **Elution of nucleic acids**		**50-100 µl of Buffer EL* (RT) carefully** **RT** **1 min** **20 000 x g 1 min (or 16 000 x g 2 min)**

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

For further information, please contact BioSellal Technical Support (tech@biosellal.com).

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