January 2023

exoEasy Maxi Kit Handbook

For purification of exosomes and other extracellular vesicles (EVs) from animal and human plasma and serum or from cell culture supernatants and other biofluids

Sample to Insight

Contents

Kit Contents

exoEasy Maxi Kit	**(20)**
Catalog no.	**76064**
Number of preps	**20**
exoEasy Maxi Spin Columns (in 50 mL Collection Tube)	20
Collection Tubes (50 mL)	20
Buffer XBP	3 x 55 mL*
Buffer XWP	2 x 200 mL
Buffer XE	45 mL
Quick-Start Protocol	1

* The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ2 Connect instruments.

Shipping and Storage

The exoEasy Maxi Kit (cat. no. 76064) is shipped at ambient temperature. Store all components dry at room temperature (15–25°C). All kit components are stable for at least 9 months upon arrival under these conditions.

To ensure compatibility with biological applications, Buffer XE is produced sterile without added preservative to prevent microbial growth. Handling under sterile conditions is recommended. Buffer aliquots can also be stored frozen to prevent growth.

Intended Use

The exoEasy Maxi Kit is intended for molecular biology applications. The product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION 	CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of exoEasy Maxi Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

In recent years, interest in the significance of RNA and other molecules carried by exosomes and other extracellular vesicles (EVs) has increased. These vesicles may be the key to identifying circulating biomarkers. Until now, methods for purifying EVs have been time-consuming, inconsistent, and often resulted in preparations contaminated by non-vesicular proteins, organic polymers, and other impurities due to the use of ultracentrifugation or non-specific precipitation reagents. The exoEasy Maxi Kit is designed for rapid purification of EVs in just 25 minutes from serum or plasma (up to 4 mL), cerebrospinal fluid (CSF; up to 8 mL), cell culture supernatant (up to 32 mL), or urine (up to 16 mL). Other cell-free biofluids have not been tested thoroughly, but may also be compatible. Although the protocol has been successfully tested for up to 32 mL of supernatant, vesicle concentration varies considerably with cell type and culture condition; therefore, it is not possible to provide a meaningful volume for binding capacity.

> **Note**: the provided amount of Buffer XBP is sufficient for processing of 20 x 8 mL samples. To process large volumes additional Buffer XBP can be ordered separately (see "Ordering Information", page 28).

Principle and procedure

The exoEasy Maxi Kit uses a membrane-based affinity binding step to isolate exosomes and other EVs from serum and plasma or cell culture supernatants. The method does not distinguish EVs by size or cellular origin and is not dependent on the presence of a particular epitope. Instead, it makes use of a generic, biochemical feature of vesicles to recover the entire spectrum of extracellular vesicles present in a sample (1). It is therefore essential to completely remove cells, cell fragments, apoptotic bodies, etc., by centrifugation or filtration of samples before starting the protocol.

Particulate matter other than vesicles, like larger protein complexes that are especially abundant in plasma and serum, is largely removed during the binding and ensuing wash step.

After washing the column membrane, intact vesicles are eluted in aqueous buffer containing primarily inorganic salts and are then ready to use for physical or biochemical analysis. For certain applications, such as biological assays, an additional concentrating or buffer exchange step may be required. This can be achieved using ultrafiltration, using a pore size of 100 kDa or smaller.

Isolated vesicles can be analyzed by and used for:

- Physical characterization (e.g., Nanoparticle Tracking Analysis (NTA), Tunable Resistive Pulse Sensing (TRPS))
- Electron microscopy
- Protein analysis
- Isolation and analysis of DNA, RNA*, lipids, and other constituents
- Flow cytometry
- Antibody- or fluorescent dye-based labeling
- Uptake by recipient cells

* For isolation of vesicular RNA, we recommend using the exoRNeasy Kits – see Ordering Information.

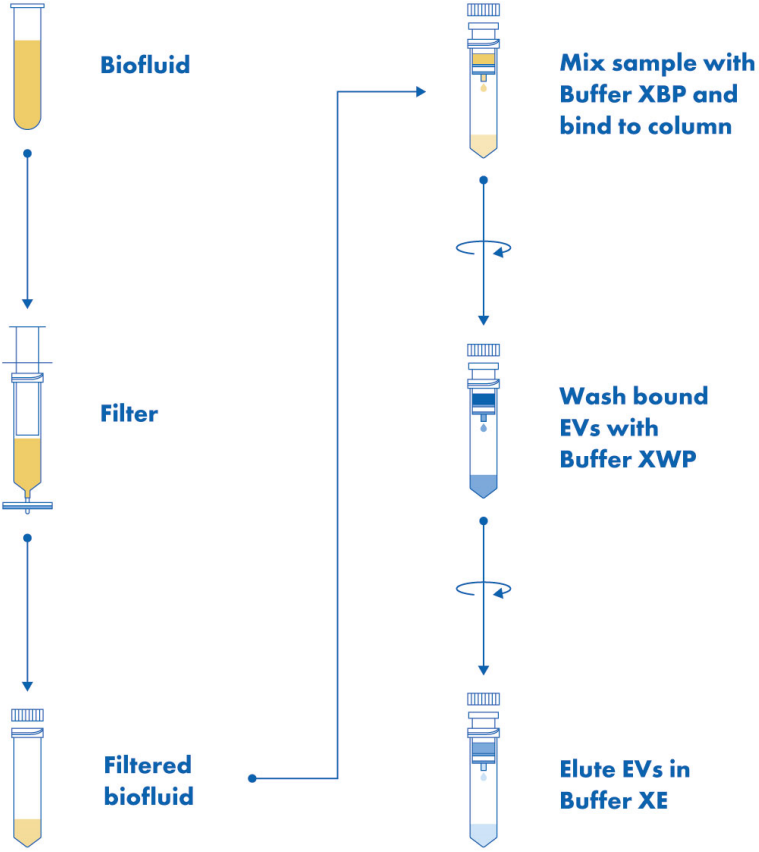

Figure 1. The exoEasy Maxi Kit allows isolation of extracellular vesicles from filtered biofluids in just 25 minutes. Eluted vesicles are ready for physical or biochemical characterization, or buffer exchange and concentration using ultrafiltration, for example.

Quality control and quantification

Quality and quantity of eluted vesicles can be determined by a range of different methods, including electron microscopy, physical characterization (such as NTA and TRPS), and analysis of molecular constituents of vesicles, such as proteins, nucleic acids, and lipids.

Since particle counting techniques like NTA and TRPS do not distinguish vesicles from other particulate matter, particle counts performed in unprocessed biofluids or crude preparations that still contain other particulate matter – such as protein complexes – may vastly overestimate the number of vesicles present.

Equipment and Reagents to Be Supplied by User

- Sterile, RNase-free pipette tips
- 1.5 mL or 2 mL microcentrifuge tubes
- Disposable gloves

Serum/plasma collection and separation (see Appendix A, page 18)

- For serum: primary blood collection tube(s) without anticoagulants such as EDTA or citrate
- For plasma: primary blood collection tube(s) containing an anticoagulant such as EDTA or citrate
- Conical tube(s)
- Refrigerated centrifuge with a swinging bucket rotor
- Syringe filters for excluding particles larger than 0.8 µm (e.g., using Sartorius® Minisart® NML (cat. no. 16592) or Millipore® Millex®-AA (cat. no. SLAA033SB))

Collection and separation of cell culture supernatants

- Conical tube(s)
- Refrigerated centrifuge with a swinging bucket rotor
- Syringe filters for excluding particles larger than 0.8 µm (e.g., using Sartorius Minisart NML (cat. no. 16592) or Millipore Millex-AA (cat. no. SLAA033SB))

Important Notes

Volume of starting material

The volume of starting material is limited by the binding capacity of the exoEasy spin column. It is not recommended to use more than 4 mL serum or plasma. For cell culture supernatants, up to 32 mL (equals 4 column loading steps) have been processed with good results. However, the concentration of vesicles in supernatants depends strongly on the cell type and culture conditions, and therefore we recommend starting with no more than 16 mL of sample for material that has not been previously tested with the kit. Higher sample volumes may result in reduced recovery of vesicles. It is strongly recommended to only use pre-filtered sample material, excluding particles larger than 0.8 µm. Filtering should be performed prior to freezing samples for storage, if possible.

> **Note**: The provided amount of Buffer XBP is sufficient for processing of 20 x 8 mL samples. To process large volumes, additional Buffer XBP can be ordered separately (see Ordering Information, page 28).

Vesicle yields can be estimated by different particle analysis technologies such as NTA and TRPS, or by quantitation of vesicular markers (e.g., proteins such as CD63 and RNA).

Table 1. exoEasy Maxi spin column specifications

Maximum volume of serum or plasma	4 mL
Maximum volume of culture supernatant	16 mL (Strongly depends on cell type and culture conditions)
Maximum column loading volume	16 mL
Allows recovery of:	Exosomes and other EVs

> **Note**: If the recommended sample volume is exceeded, recovery of vesicles will not be consistent and may be reduced.

Protocol: Purification of Exosomes and other Extracellular Vesicles from Serum and Plasma or from Cell Culture Supernatants Using the exoEasy Maxi Kit

This protocol is for purifying exosomes and other EVs from 0.2–4 mL of serum or plasma, or from up to 16 mL of cell culture supernatant. However, the binding capacity for cell culture supernatants may vary strongly with cell type and culture conditions.

For recommendations on collection, preparation and storage of cell-free plasma and serum, see Appendix A, page 18.

For recommendations on culturing cells and harvesting of culture supernatants, see Appendix B, page 21.

Important points before starting

- The procedure is suitable for use with either serum or plasma samples containing citrate or EDTA. Plasma samples containing heparin are not recommended because this anticoagulant is difficult to remove and can interfere with downstream applications based on enzymatic reagents.
- For isolation of EVs from cell culture supernatants, it is essential to use either serum-free medium or medium prepared with vesicle-free serum.
- After collection and centrifugation, plasma, serum, or cell culture supernatant can be stored at 2–8°C for up to 6 h or used directly in the procedure. For long-term storage, freezing in aliquots at –15°C to –30°C or –65°C to –90°C is recommended. To process frozen samples, incubate at 37°C in a water bath until samples are completely thawed. Avoid prolonged incubation as this may compromise integrity of EVs.

- All steps should be performed at room temperature (15–25°C). Carry out the protocol steps quickly but carefully.
- Centrifugation of the exoEasy Maxi spin columns is performed in a standard laboratory centrifuge with a swinging bucket rotor, preferably capable of up to 5000 x *g* (it is possible to reduce the steps performed at 5000 x *g* down to a minimum force of 3000 x *g* without performance loss).

Procedure

1. It is recommended to use only pre-filtered serum, plasma, or cell culture supernatant. Supernatants should be filtered to exclude particles larger than 0.8 µm (e.g., using Sartorius Minisart NML (cat. no. 16592) or Millipore Millex-AA (cat. no. SLAA033SB) syringe filters).

   Important: For cell culture supernatants, filtering should be performed prior to freezing of samples.

2. Add 1 volume buffer XBP to 1 volume of sample. Mix well by gently inverting the tube 5 times. Let the mixture warm up to room temperature.

3. Add up to 16 mL of the sample/XBP mix onto the exoEasy spin column and centrifuge at 500 x *g* for 1 min. Discard the flow-through and place the column back into the same collection tube.

   If the sample volume is greater than 8 mL, repeat this step until the entire volume has been passed through the column.

   Optional: To remove residual liquid from the membrane, centrifuge at 5000 x *g* for 1 min.

4. Add 10 mL buffer XWP and centrifuge at 5000 x *g* for 5 min to remove residual buffer from the column. Discard the flow-through together with the collection tube.

   Note: It is possible to reduce the centrifugation speed from 5000 x *g* down to a minimum force of 3000 x *g* without loss of performance.

   Optional: Repeat step 4 to further reduce nonspecifically bound materials (e.g., free proteins). Reuse the collection tube from step 4.

5. Transfer the spin column to a fresh collection tube.
6. Add 250–400 µL Buffer XE to the membrane and incubate for 1 min. Centrifuge at 500 x *g* for 5 min to collect the eluate.

   Note: Using less than 250 µL elution buffer will result in incomplete elution. Eluates can be concentrated (e.g., using ultrafiltration, (see Appendix D, page 25)). If an ultrafiltration step will be performed, eluting in 1–2 mL Buffer XE is recommended.

   Optional: Re-apply the eluate to the exoEasy spin column membrane and incubate for 1 min. Centrifuge at 5000 x *g* for 5 min to collect the eluate and transfer to an appropriate tube (not supplied).

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
Clogged exoEasy column	
a) Sample still contains cellular material or coagulated protein	Make sure to follow recommendations in Appendix A (page 18) for removal of residual cellular material. After thawing of frozen samples, remove cryoprecipitates by centrifugation or filtration, if necessary (see Appendix A).
Low particle count	
a) Comparison to vesicle preparations contaminated by other particulate matter	Vesicles isolated by ultracentrifugation or especially by precipitation reagents usually contain non-vesicular particulate matter, like larger protein complexes, lipoprotein complexes, etc., as well. These contaminants can result in inflated particle counts that are not representative of EV concentration.
Isolation of vesicles other than exosomes	
Recovery of all types of EVs	The exoEasy technology is designed and developed to isolate all types of EVs, not just exosomes. It does not distinguish vesicles by size, cellular origin or the presence of particular proteins or epitopes. It is possible to exclude larger vesicles by modification of the initial filtration or centrifugation step.

References

1. Enderle D, Spiel A, Coticchia CM, Berghoff E, Mueller R, Schlumpberger M, et al. Characterization of RNA from Exosomes and Other Extracellular Vesicles Isolated by a Novel Spin Column-Based Method. *PLoS ONE* 2015; 10(8): e0136133. **doi:10.1371/journal.pone.0136133**.
2. Shelke GV, Lasser C, Gho YS, Lötvall J. Importance of exosome depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. *Extracell Vesicles* 2014; 3(1) 24783 **http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4185091/#CIT0013**.

Appendix A: Recommendations for Serum and Plasma Collection, Separation, and Storage

In order to specifically isolate EVs from serum or plasma, we recommend including an initial low *g*-force centrifugation step to separate cells from plasma or serum, followed by a higher *g*-force centrifugation or filtration step to remove all remaining cellular debris. The latter centrifugation/filtration step reduces the number of particles in the sample, but is essential to avoid contamination by cell fragments, apoptotic vesicles, etc. The removal of cellular materials should be performed immediately after blood collection to lower the risk of additional background from blood cell-derived vesicles generated in vitro. Use of gel barrier tubes generally results in fewer residual cells.

Because binding of vesicles to the exoEasy membrane is not selective for a specific sub-population or size range of EVs, the filtration or centrifugation step performed at this stage can also influence the size range of vesicles that will be isolated.

> **Note**: The recommended 0.8 µm filter pore size (or centrifugation at 3,000 x *g*) will effectively exclude cellular material, including thrombocyte fragments, but still retains the vast majority of EVs, whereas use of a 0.2 µm or 0.45 µm filter (or centrifugation at 16,000 x *g*) will remove some of the larger vesicles.

The recommended syringe filters (e.g., Sartorius Minisart NML (cat. no. 16592) or Millipore Millex-AA (cat. no. SLAA033SB)) have dead volumes of around 100–200 µL. Small volumes of sample (<1 mL) can be diluted with PBS prior to filtration to reduce loss of material.

Procedure: Plasma separation and storage

1. Collect whole blood in BD Vacutainer® Venous Blood Collection Tubes (cat. no. 367525) containing EDTA (or any other primary blood collection tube containing an anticoagulant such as EDTA or citrate). Store tubes at room temperature (15–25°C) or 4°C and process within 1 h.

   Note: Do not use heparin-containing blood collection tubes as this anticoagulant is difficult to remove and can interfere with downstream applications.

2. Centrifuge blood samples in primary blood collection tubes for 10 min at 3000 rpm and 4°C using a swinging bucket rotor.
3. Carefully transfer the upper (yellow) plasma phase to a new tube (with conical bottom) without disturbing the intermediate buffy coat layer (containing white blood cells and platelets). Normally up to 4–5 mL plasma can be obtained from 10 mL whole blood.
4. Centrifuge plasma samples in conical tubes for 15 min at 3,000 x *g* (or 10 min at 16,000 x *g* — see Note in page 18) and 4°C or pass through a 0.8 µm filter (see "Equipment and Reagents to Be Supplied by User"). This will remove additional cellular fragments and cell debris.
5. Carefully transfer cleared supernatant to a new tube without disturbing the pellet, which forms a smear along the outer side/bottom of the centrifugation tube.
6. Store at 2–8°C until further processing if plasma will be used for isolation of EVs on the same day. For longer storage, keep plasma frozen in aliquots at –65°C to –90°C.
7. Before using frozen plasma for isolation of EVs, thaw at room temperature (15–25°C).

   Optional: To remove cryoprecipitates, centrifuge thawed plasma samples for 5 min at 3,000 x *g* and 4°C or pass through a 0.8 µm filter. Transfer supernatant to a new tube, and begin EV isolation protocol.

Procedure: Serum separation and storage

1. Collect whole blood in a primary blood collection tube with or without clot activator, but without anticoagulants such as EDTA or citrate (e.g., Sarstedt® S-Monovette® Serum Gel 9 mL tubes, cat. no. 02.1388). For complete clotting, leave tubes at room temperature (15–25°C) for 10 min to 1 h.

   Note: Tubes with clot activator can be processed after 10 min clotting time, while tubes without clot activator should be stored for at least 30 min at room temperature to allow clotting to take place.

2. Centrifuge tubes for 10 min at 3000 rpm and 4°C using a swinging bucket rotor.

   Note: If using Sarstedt S-Monovette Serum Gel 9 mL tubes, a gel bed will form between the upper serum phase and the lower cellular phase, facilitating recovery of serum.

3. Carefully transfer the upper (yellow) serum phase to a new tube (with conical bottom) without disturbing the pellet containing cellular material. Normally up to 3–5 mL serum can be obtained from 10 mL of whole blood.

   Note: Prevent transfer of cellular material from the lower phase.

4. Centrifuge serum samples in conical tubes for 15 min at 3,000 x *g* (or 10 min at 16,000 x *g*, see Note in page Appendix A: Recommendations for Serum and Plasma Collection, Separation, and Storage) and 4°C or pass through a 0.8 µm filter (see "Equipment and Reagents to Be Supplied by User"). This will remove additional cellular fragments and cell debris.

5. Carefully transfer cleared supernatant to a new tube without disturbing the pellet, which forms a smear along the outer side of the centrifugation tube.

6. Store at 2–8°C until further processing if serum will be used for isolation of EVs on the same day. For longer storage, keep serum frozen in aliquots at –65°C to –90°C.

7. Before using frozen serum for isolation of EVs, thaw at room temperature (15–25°C).

   Optional: To remove cryoprecipitates, centrifuge thawed serum samples for 5 min at 3,000 x *g* and 4°C or pass through a 0.8 µm filter. Transfer supernatant to a new tube and begin EV isolation protocol.

Appendix B: Recommendations for Culturing Cells and Harvesting of Culture Supernatants

Growth medium

Probably the most important aspect in cultivating cells for isolation of EVs is making sure that no extrinsic vesicles are introduced by the growth medium. Fetal calf (or bovine) serum (FCS) commonly used in growth media contains bovine EVs that need to be excluded. This can be achieved by transferring cells to serum-free medium for production of vesicles, or to medium prepared with vesicle-free serum. Vesicle-free serum is typically prepared by ultracentrifugation at 120,000 × *g* for 18 hours (2), but is also commercially available. We recommend performing 2 or 3 washes with PBS or vesicle-free medium to completely eliminate EVs of bovine origin.

Preparation of culture supernatants

In order to specifically isolate EVs from cell culture supernatants, we recommend including an initial low *g*-force centrifugation step to separate detached cells from growth medium, followed by a higher *g*-force centrifugation or filtration step to remove all remaining cellular debris. The latter centrifugation/filtration step reduces the number of particles in the sample, but is essential to avoid contamination by cell fragments, apoptotic vesicles, etc.

Because binding of vesicles to the exoEasy membrane is not selective for a specific sub-population or size range of EVs, the filtration or centrifugation step performed at this stage can also influence the size range of vesicles that will be isolated. The recommended 0.8 µm filter pore size (or centrifugation at 3,000 x *g*) will effectively exclude cellular material, but still retains the vast majority of EVs, whereas use of a 0.2 µm or 0.45 µm filter (or centrifugation at 16,000 x *g*) will remove some of the larger vesicles.

The recommended syringe filters (e.g., Sartorius Minisart NML (cat. no. 16592) or Millipore Millex-AA (cat. no. SLAA033SB)) have dead volumes of around 100–200 µL.

In order to minimize contaminants generated by dying cells (apoptotic bodies, intracellular vesicles, free RNA, and DNA, among others), we recommend avoiding conditions that may lead to increased cell death, such as growing cells to confluency or beyond, etc.

Procedure

1. Aspirate culture supernatant and collect in an appropriately sized tube.
2. Centrifuge in conical tubes for 15 min at 3,000 x *g* (or 10 min at 16,000 x *g*, see Note in page 18) and 4°C or pass through a 0.8 µm filter (see "Equipment and Reagents to Be Supplied by User"). This will remove additional cellular fragments and cell debris.
3. Carefully transfer cleared supernatant to a new tube without disturbing the pellet, which forms a smear along the outer side of the centrifugation tube.
4. Store at 2–8°C until further processing if supernatant will be used for isolation of EVs on the same day. For longer storage, keep frozen in aliquots at –65°C to –90°C.
5. Before using frozen culture supernatants for isolation of EVs, thaw at room temperature (15–25°C).

   Optional: To remove cryoprecipitates, centrifuge thawed samples for 5 min at 3,000 x *g* and 4°C or pass through a 0.8 µm filter. Transfer supernatant to a new tube and begin EV isolation protocol.

RNase treatment of cell culture supernatant

In cases where RNA isolation from CCS – specifically of RNA from inside EVs – is intended, we recommend treating the samples with RNase to eliminate any RNA released by cells independent of vesicles (as a result of cell death, for example). In our experience, RNA released in such ways is often in orders of magnitude more abundant than RNA inside EVs,

so even if small proportions of that RNA are co-isolated with RNA from EVs, it can very strongly affect results.

We find the RNase treatment described here to be very efficient in eliminating free RNA, whereas RNA inside EVs is not affected. The added RNase is efficiently removed during RNA isolation – e.g., using the miRNeasy Serum/Plasma Kit (see "Appendix D: Use of Eluates in Downstream Applications").

Supplied by user: RNase A (100 mg/mL), QIAGEN cat. no. 19101 (2.5 mL, 7000 units/mL)

1. Prepare 10 mg/mL working solution, add 2.5 µL per 1 mL CCS (final conc.: 25 µg/mL).
2. Mix and incubate at room temperature (15–25°C) for 20 min.
3. Proceed with the exoEasy protocol.

Appendix C: Use with Other Biofluids

The exoEasy Maxi Kit has also been used for isolation of extracellular vesicle from diverse starting material like urine, cerebrospinal fluid, saliva, sweat, milk, synovial fluid, etc. No thorough testing or dedicated protocol development has been done by QIAGEN so far, however. Depending on samples type there may be additional contaminants present.

Appendix D: Use of Eluates in Downstream Applications

Although the exact composition of Buffer XE is proprietary, the main constituents are inorganic salts. To ensure compatibility with biological applications, Buffer XE is produced sterile without added preservative to prevent microbial growth. Handling under sterile conditions is recommended. Buffer aliquots can also be stored frozen to prevent microbial growth.

For experiments where isolated EVs are taken up by target cells to elicit a biological response, exoEasy eluates can either be diluted into cell culture medium directly, or subjected to a buffer exchange step first (e.g., by ultrafiltration, see Note below), in order to ensure full compatibility of the vesicle suspension with cell culture. As a rough guideline, when 5% of buffer XE were added to HeLa cells grown in DMEM (125 µL in 2.5 mL medium) for up to 24 hours, no change in cell morphology, density, or vitality (Trypan Blue stain) was observed. Whenever vesicles in buffer XE are added to cells directly, we strongly recommend to perform a mock control with buffer XE alone.

> **Note**: For applications requiring more concentrated vesicle preparations, lower salt concentration, or specific buffer conditions, ultrafiltration can be used to reduce eluate volume and exchange buffer conditions. Sartorius Vivaspin® 500 and Vivaspin 2 with molecular weight cut-off of 100 kDa or below (e.g., Sartorius cat. no. VS0141, or VS02X1) have been used successfully according to manufacturer's recommendations.

Buffer exchange without volume reduction can also be achieved using dialysis or size exclusion chromatography columns. For dialysis the Slide-A-Lyzer™ MINI Dialysis Devices (100-500 µL) (Thermo Fisher) can be used.

RNA isolation

RNA can be isolated from exoEasy eluates using the miRNeasy Serum/Plasma Kit, following the standard protocol for plasma samples. Please note that miRNeasy Serum/Plasma Advanced is not recommended for this application.

However, unless there are specific reasons to isolate intact EVs first (e.g., using some of the eluate for other purposes), we recommend using the exoRNeasy Maxi or Midi Kit instead, since it provides a more streamlined protocol and already contains all of the required reagents.

Protein analysis

To determine total protein concentration in exoEasy eluates (i.e., in Buffer XE), the Pierce™ BCA Protein Assay Kit (Thermo Fisher) can be used according to manufacturer's instructions. No extra steps for EV lysis need to be performed. Similarly, exoEasy eluates can be used in standard SDS-PAGE and Western Blotting protocols without any pretreatment.

For optimal results and convenience, we recommend sensitive and automated protein separation and immunodetection on the Simple Western™ platforms (e.g., Jess™) from ProteinSimple, a Bio-Techne brand (**https://www.bio-techne.com/instruments/simple-western**).

For non-targeted proteomics applications, in case of EV eluates from plasma or serum, the presence of residual amounts of plasma proteins needs to be taken into consideration. While the exoEasy procedure removes >99% of albumin and other highly abundant proteins, the remaining amounts typically still exceed the amount of EV proteins. Similar considerations apply for EV eluates obtained from other complex biofluids with high protein content.

For antibody-based detection under native conditions – e.g., staining for (high resolution) fluorescence microscopy, or flow cytometry – the salt concentration in buffer XE may be high

enough to affect binding of antibodies. Performing a buffer exchange step (see page 25) or diluting eluates 5-fold or more should be sufficient to avoid any such problems.

EM analysis

For negative staining-based procedures using carbon grids, eluates either need to be diluted at least 5-fold or a buffer exchange (see page 25) should be performed.

Ordering Information

Product	Contents	Cat. no.
exoEasy Maxi Kit (20)	For 20 vesicles preps: 20 exoEasy Maxi Spin Columns, Collection Tubes (50 mL), Reagents and Buffers	76064
Buffer XBP (250 mL)	250 mL Binding Buffer	76204
exoRNeasy Maxi Kit (50)	For 50 RNA preps: 50 exoEasy Maxi and RNeasy® MinElute Spin Columns, Collection Tubes (1.5 mL, 2 mL, and 50 mL), QIAzol® Lysis Reagent, RNase-free Reagents and Buffers	77164
exoRNeasy Midi Kit (50)	For 50 RNA preps: 50 exoEasy Midi and RNeasy MinElute Spin Columns, Collection Tubes (1.5 mL, 2 mL, and 50 mL), QIAzol Lysis Reagent, RNase-free Reagents and Buffers	77144
exoRNeasy Starter Kit (20)	For 20 RNA preps: 10 exoEasy Maxi, 10 exoEasy Midi and 20 RNeasy MinElute Spin Columns, Collection Tubes (1.5 mL, 2 mL, and 50 mL), QIAzol Lysis Reagent RNase-free Reagents and Buffers	77023

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Document Revision History

Revision	Description
06/2015	Initial release
01/2023	Updated protocol to include an additional wash step. Appendix B now includes discussion on RNase treatment of cell culture supernatant. Added Appendix C. Updated Appendix D to cover RNA isolation and protein analysis.

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