Capturem™ Extracellular Vesicle Isolation Kit (Mini) Protocol-At-A-Glance

I. Introduction

This protocol is provided for the Capturem Extracellular Vesicle Isolation Kit (Mini) (Cat. No. 635741), which is designed for the easy and rapid isolation of extracellular vesicles (EVs) and **yields up to 10^{10} purified EVs per column**. This kit is compatible with various biological fluids, such as plasma, serum, urine, milk, saliva, and cell-conditioned media.

II. Materials and Reagents

A. Components

- 20 ea. Capturem Extracellular Vesicle Isolation Mini Spin Columns (with green inserts) in 2-ml collection tubes
- 20 ea. Extracellular Vesicle Isolation Pre-Clearing Columns (with purple inserts) in 2-ml collection tubes
- 20 ml Extracellular Vesicle Isolation Equilibration Buffer
- 20 ml Extracellular Vesicle Isolation Wash Buffer
- 4 ml Extracellular Vesicle Isolation Elution Buffer

B. Additional Materials Required

- 20 ea. Amicon® Ultra-0.5 Centrifugal Filter Unit, 100-KDa MWCO
- Collection tubes: Each isolation will require three additional standard 2-ml collection tubes suitable for centrifugation up to 3,000*g*. These tubes should be used throughout the protocol to collect flowthrough, wash, and eluate samples.
- PBS without $CaCl_2$ or $MgCl_2$

III. General Considerations

- Sample supernatant must be prefiltered using the supplied Extracellular Vesicle Isolation Pre-Clearing Column to remove any large membrane fragments, apoptotic bodies, smaller cell fragments, etc., to avoid any possible blocking of the Capturem Extracellular Vesicle Isolation Columns.
- After preclearing, Amicon Ultra-0.5 Centrifugal Filter Units, 100-KDa MWCO are used to remove nonspecific protein aggregates, lipoproteins, and cytokines, etc. before isolation, resulting in more highly purified EVs.

NOTE: Proteinase K treatment can also be used to remove most of the protein from samples, but it may result in partial degradation of proteins exposed on the surface of the EVs.

- This protocol elutes EVs using a phosphate-based buffer containing organic salts. A desalting step may be required for some downstream applications, such as protein and RNA extraction (see Section V below).
- The recommended starting volume* for biological samples is listed below:

Sample	Recommended volume
Plasma, serum, milk, and saliva	<500 µl
Urine, cell-conditioned media	>500 µl

*Since each column can bind a maximum of 10^{10} EVs, overloading the column will not increase the yield beyond this point. For larger sample volumes, we suggest using multiple columns.

IV. Protocols

A. Collecting Sample Supernatant

Each column can hold up to 850 µl of sample supernatant. If your sample type is known to have a high concentration of EVs, it is prudent to use less than this full volume and dilute with PBS (see Step 4). **As each column will max out at 10^{10} EVs, overloading the column will not increase yield beyond this point.**

1. Thaw frozen samples in a room-temperature water bath until completely liquid.
2. Centrifuge at 3,000*g* for 10 min to remove any cellular debris.
3. Transfer the supernatant to a new collection tube (supplied by the user).
4. Dilute your supernatant with PBS to a volume of 850 µl. **If you anticipate that your sample will already have a low concentration of EVs, it is not necessary to dilute the supernatant.**
5. Load the supernatant onto the Capturem Extracellular Vesicle Pre-Clearing column and centrifuge at 3,000*g* for 2 min at room temperature or longer, until all the solution passes through the columns. Save the collection tube containing the sample flowthrough for downstream analysis.

   NOTE: Highly viscous samples may clog the Pre-Clearing column. If this occurs, collect the resulting flowthrough and proceed to Step 6. Be aware that this may result in lower yield, as some EVs may remain in the clogged column**.**
6. Load the flowthrough from Step 5 onto an Amicon Ultra-0.5 Centrifugal Filter Unit, 100-KDa MWCO and concentrate to ≤100 µl by repeated centrifugation. Collect the concentrate, then wash the filter membrane with 100 µl PBS, and add to the concentrate. Adjust the sample volume to 850 µl with PBS.

   NOTE: Highly viscous samples may clog the Amicon Ultra-0.5 Centrifugal filter units. If this occurs, collect the concentrate and wash the filter membrane with PBS, then adjust the sample volume to 850 µl with additional PBS and proceed to Section B. Be aware that this may result in lower yields, as some EVs may remain in the clogged filter unit.

B. Extracellular Vesicle Isolation

1. Equilibrate a Capturem Extracellular Vesicle Isolation Column with 0.5 ml Extracellular Vesicle Isolation Equilibration Buffer. Centrifuge at 3,000*g* for 2 min at room temperature or longer, until all solution passes through the column. Discard the flowthrough.

   NOTE: Extracellular Vesicle Isolation Equilibration Buffer contains ions which will interfere with Pierce BCA Protein Assay Kits, Bradford protein assays, NanoDrop readings, etc.
2. Load the pre-filtered supernatant (850 µl) onto the equilibrated Capturem Extracellular Vesicle Isolation Column that has been placed in a new collection tube (supplied by the user). Centrifuge at 500*g* for 2 min at room temperature or longer, until all solution passes through the columns.
3. Place the Capturem Extracellular Vesicle Isolation Column in a new collection tube (supplied by the user) and add 0.5 ml of Extracellular Vesicle Isolation Wash Buffer to the spin column. Centrifuge at 500*g* for 2 min at room temperature or longer, until all solution passes through the columns.
4. Insert the Extracellular Vesicle Isolation Column into a new collection tube (supplied by the user) and add 100 µl of Extracellular Vesicle Isolation Elution Buffer to the column. Centrifuge at 500*g* for 2 min at room temperature to collect the eluate.

5. Repeat the elution by reloading the eluate from Step 4 onto the same Extracellular Vesicle Isolation Column and centrifuging at 500*g* for 2 min at room temperature to collect the final eluate.
6. Eluted extracellular vesicles are now ready for downstream applications and analysis. See Section V for important information on storage and use.

V. Considerations for Downstream Applications and Analyses

A. Storage

Eluted extracellular vesicles can be stored at 4°C for up to 1 week. We recommend –20°C or –80°C for long-term storage. To avoid any damage or loss due to repeated freeze/thaw cycles, we recommend aliquoting them into multiple tubes and only thawing the amounts needed for a given application.

B. Preparation for Downstream Applications and Analysis

The Capturem Extracellular Vesicle Isolation Kit (Mini) protocol is a much gentler process than traditional ultracentrifugation, and as such, minimizes potential damage to the EVs.

If performing an analysis of protein content in the eluate, note that it will be necessary to look for markers specific to extracellular vesicles, as this protocol removes the bulk of proteins not specific to EVs, including carryover proteins like albumin.

This protocol elutes EVs using a phosphate-based buffer containing organic salts. Eluted particles can be directly used for physical particle analysis, such as nanoparticle tracking analysis (NTA), and labeling for cellular uptake assays. **If your downstream application involves protein and/or RNA extraction, it will be necessary to desalt the eluted samples**. We recommend the following steps:

1. Load the entire sample on an Amicon Ultra 0.5-ml centrifugal filter (either 3-kD or 10-kD MWCO, supplied by the user). Add water to the filter unit to bring sample volume up to 500 µl. Centrifuge at 14,000*g* for 5–30 min and discard the flowthrough.
2. Add water to the filter unit to bring the sample volume up to 500 µl, and centrifuge at 14,000*g* for 5–30 min.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com	web: takarabio.com
e-mail: ordersUS@takarabio.com	e-mail: technical_support@takarabio.com

Notice to Purchaser

This document has been reviewed and approved by the Quality Department.