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). The workflow (Figure 1) is a much gentler process than traditional ultracentrifugation, therefore minimizing potential damage to the EVs. This kit is compatible with various biological fluids, such as plasma, serum, urine, milk, saliva, and cell-conditioned media, and yields up to 10^{10} purified EVs per column.

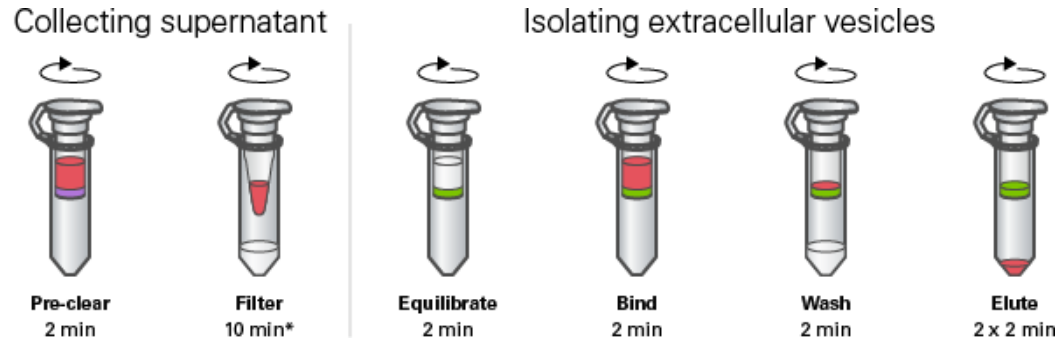


Figure 1. The Capturem Extracellular Vesicle Isolation Kit (Mini) workflow can be completed in 30 minutes. *The Filter step uses a filter unit with a 100-kDa molecular weight cutoff (not included). Filtration time will vary depending on sample type and volume.

II. Materials and Reagents

A. Components

The following components are included in the kit:

- 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

The following materials are required but not supplied:

- 20 ea. Amicon Ultra-0.5 Centrifugal Filter Unit, 100-KDa MWCO (Thermo Fisher Scientific, Cat. No. UFC510024)
- Collection tubes: two additional tubes will be required in Section IV.A to collect and transfer the sample supernatant. Three additional standard 2-ml collection tubes will be required in Section IV.B to collect the flowthrough, wash, and eluate samples. These tubes should be suitable for centrifugation up to 3,000*g*.
- PBS without $CaCl_2$ or $MgCl_2$

III. General Considerations

Samples must be pretreated before loading into Capturem Extracellular Vesicle Isolation Mini Spin Columns. First, the sample supernatant must be pre-cleared using the supplied Extracellular Vesicle Isolation Pre-Clearing Columns to remove any large membrane fragments, apoptotic bodies, smaller cell fragments, etc. After pre-clearing, an Amicon Ultra-0.5 Centrifugal Filter Unit, 100 kDa MWCO is used to remove nonspecific protein aggregates, lipoproteins, cytokines, etc., resulting in EVs of higher purity. Alternatively, Proteinase K treatment can be used to remove the bulk of the protein from samples, but it may result in partial degradation of proteins exposed on the surface of the EVs. After filtering, samples are loaded into Capturem extracellular vesicle isolation columns at the recommended loading volumes (see Table I).

Table I. Recommended loading volumes for biological samples

Sample*	Recommended column loading volume
Plasma, serum, milk, or saliva	<500 µl
Urine or cell-conditioned media	>500 µl

*Since each column can bind a maximum of 10^{10} EVs, overloading the column will not increase yield beyond this point. These are general guidelines/recommendations based on our observations. The ideal/optimal loading volume may vary depending on your sample source. For larger-volume samples, please use Capturem Extracellular Vesicle Isolation Kit (Maxi) (Cat. No. 635748).

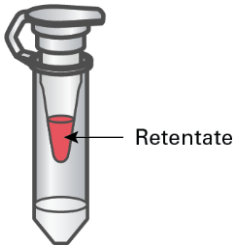
IV. Protocols

A. Collecting Sample Supernatant

1. Thaw the frozen sample in a water bath at room temperature 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. Load the supernatant onto the Capturem Extracellular Vesicle Isolation Pre-Clearing Column and centrifuge at 3,000*g* for 2 min at room temperature, or until all the solution passes through the column. This time may need to be extended for highly viscous samples or larger volumes. Save the collection tube containing the sample flowthrough for downstream analysis.

   NOTE: A highly viscous sample may get clogged in the column. To prevent clogging, we recommend diluting highly viscous samples (like plasma and serum) 1:3 with PBS. If clogging still occurs, collect the resulting flowthrough and proceed to Step 5. Be aware that this may result in lower yield, as some EVs may remain clogged in the column.

5. Load the flowthrough from Step 4 onto an Amicon Ultra-0.5 centrifugal filter unit. Repeatedly centrifuge at 3,000*g* until retentate volume is ≤100 µl. Transfer the retentate to a new 2-ml collection tube (supplied by the user). Add 100 µl of PBS into the filter, and then wash off the remaining EVs by gently pipetting the PBS onto the sides of the filter a few times. Combine this supernatant with the already collected retentate. Adjust this sample volume to 800 µl with PBS.



   NOTE: A highly viscous sample may get clogged in the filter units. If this occurs, collect the retentate and the supernatant from washing the filter membrane with PBS, then adjust the sample volume to 800 µl with additional PBS and proceed to Section B. Be aware that this may result in lower yield, as some EVs may remain clogged in the column.

B. Isolating Extracellular Vesicles

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, until all solution passes through the column. Discard the flowthrough.

   NOTE: Extracellular Vesicle Isolation Equilibration Buffer contains ions that will interfere with ion-absorbance-based applications like Pierce BCA Protein Assay Kits, Bradford protein assays, NanoDrop readings, etc. However, the buffer is suitable for assays that are noncolorimetric or that do not involve ion absorbance, such as nanoparticle tracking analysis (NTA).
2. Load the filtered supernatant (800 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, until all solution passes through the column. This time may need to be extended for highly viscous samples or larger volumes.
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 column. Centrifuge at 500*g* for 2 min at room temperature, until all solution passes through the column. This time may need to be extended for highly viscous samples or larger volumes.
4. Insert the Capturem 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 Capturem 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. Storing Isolated Extracellular Vesicles

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 the eluate into multiple tubes and only thawing the amounts needed for a given application.

B. Preparing for Downstream Applications and Analysis

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 NTA and labeling for cellular uptake assays. If your downstream application involves protein and/or RNA extraction, it will be necessary to first sonicate or desalt the eluted samples. Please see our FAQs for recommended steps and additional information about the EV isolation protocol.

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