Thermo Fisher SCIENTIFIC

Exosome isolation

Rapid bead-based isolation of exosomes for multiomic research

Keywords

Dynabeads, strong anion exchange (SAX), KingFisher Flex, KingFisher Duo Prime, KingFisher Apex, automation, Spectradyne, exosomes, enrichment, isolation, purification, western blot, magnetic beads, CD81, release, flow cytometry, electron microscopy, mass spectrometry, immunolabeling, negative stain, serum, extracellular vesicles (EVs), proteomics, multiomics, liquid biopsy

In this application note, we show:

- A bead-based solution for rapid isolation of exosomes for multiomic research
- How magnetic bead–based isolation from serum provides greater yields of exosomes
- A simple, fast, and reliable method for the isolation of exosomes for manual or automated handling

Introduction

Cancer is a complex and heterogeneous disease that poses a significant public health burden worldwide. While considerable progress has been made in understanding the molecular mechanisms underlying cancer development and progression, much remains unknown. Advancements in research and diagnosis are critical for reducing its impact. Liquid biopsy is a rapidly evolving field that provides a noninvasive method for diagnosing and monitoring diseases and offers a promising approach for further advancement of cancer research. Unlike traditional tissue biopsies, liquid biopsy involves the analysis of bodily fluids to detect circulating biomarkers such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomes that reflect the presence and progression of disease.

Exosomes are 30–150 nm extracellular vesicles (EVs) containing cargo of bioactive molecules, such as RNA and protein, and are observed in most body fluids. They play an important role in intercellular communication by transferring bioactive molecules between cells, their cargo reflecting the physiological and pathological status of the parent cells. By analyzing the cargo of exosomes isolated from liquid biopsies, researchers can gain insight into cancer progression and drug resistance, as well as facilitation of the immune response, antigen presentation, programmed cell death, angiogenesis, inflammation, coagulation, dissemination of oncogenes from tumor cells, and removal of obsolete molecules.

Several methods are commonly used for exosome isolation, such as ultracentrifugation, precipitation, size exclusion, and magnetic beads. Identification of the isolated exosomes often relies on a combination of methods, including electron microscopy for ultrastructural analysis by size, and concentration distribution measurements by, for example, light scattering–based methods (dynamic light scattering and nanoparticle tracking analysis) in combination with flow cytometry, RT-qPCR, and western blotting.

invitrogen

Here we present a method for generic exosome isolation within 10 minutes using Invitrogen™ Dynabeads™ Intact Virus Enrichment magnetic beads. A panel of different methods was used to identify the isolated exosomes. Measurements of vesicle size and concentration were done by microfluidic resistive pulse sensing analysis. Analysis with the Spectradyne™ ARC™ Particle Analyzer, western blot, electron microscopy, mass spectrometry, and flow cytometry methods were used for extensive characterization of the isolated exosomes. Finally, we demonstrate isolation of exosomes from serum samples.

Fast and simple exosome isolation

We describe a manual workflow for isolation of intact exosomes from cell culture medium, performed in 10 minutes with an option to release the exosomes from the beads in another 10 minutes. This short and simple isolation approach reduces the risk of low yield and does not affect the integrity of the exosomes. One of the key features of Invitrogen™ Dynabeads™ magnetic beads used in any isolation protocol is the rapid binding kinetics of the beads. The proximity of the beads to the targets in the solution translates directly to short incubation times and therefore fast protocols (Figure 1A). Here we take advantage of the negative charge of the exosomes (Figure 1B) combined with the rapid binding kinetics of the Dynabeads magnetic beads. Positively charged Dynabeads Intact Virus Enrichment beads bind to negatively charged exosomes, viruses, or proteins within 10 minutes (Figure 2A). Following capture, the exosomes (or other negatively charged vesicles) are released from the beads in 10 minutes by adding an anion with a stronger relative affinity than the bound vesicle. This short and easy isolation approach can be simplified even further by using the Thermo Scientific™ KingFisher™ Purification System (Figure 2B). The automated isolation method allows larger numbers of samples to be processed in only 10–20 minutes with high reproducibility, reduced hands-on time, and minimal error rates.

Overview of manual and automated protocols

Exosomes derived from SW480 cells were spiked into cell culture medium, and then isolated with Dynabeads Intact Virus Enrichment beads using either a manual (Figure 2A) or automated protocol (Figure 2B). Automated isolation was performed on the Thermo Scientific™ KingFisher™ Duo Prime instrument. Alternatively, the Thermo Scientific™ KingFisher™ Flex or Apex instrument can be used.

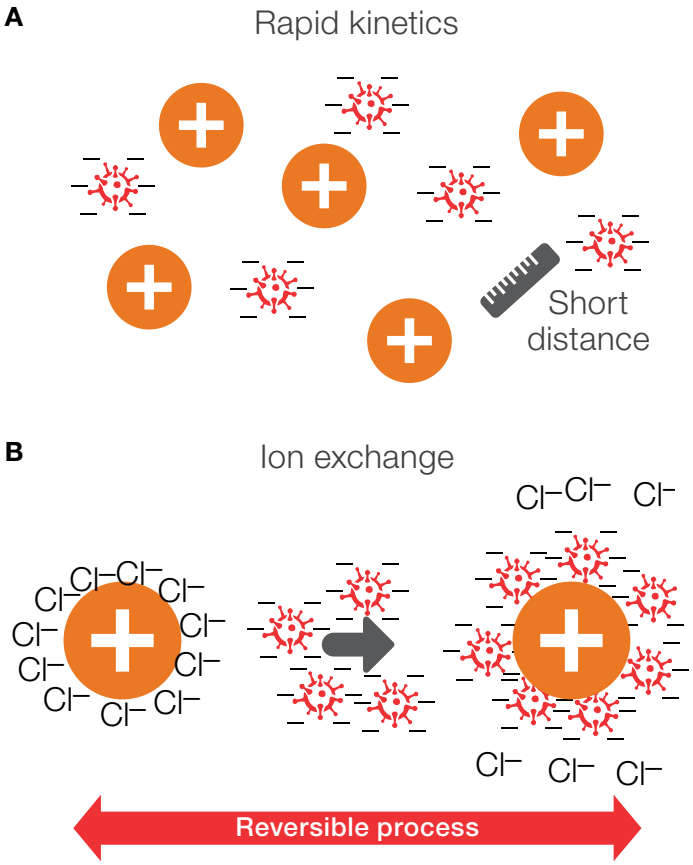

Figure 1. Isolation principle. (A) The positively charged Dynabeads Intact Virus Enrichment beads are near the negatively charged exosomes, enabling rapid binding kinetics and a fast isolation protocol. **(B)** For isolation of negatively charged exosomes or viruses, positively charged Dynabeads Intact Virus Enrichment beads protected with Cl^- ions are used. Exosomes added to the Dynabeads Intact Virus Enrichment beads will replace the Cl^- ions and bind to the bead surface. An anion with higher relative affinity can subsequently be added to replace the exosomes and thus release them into the sample.

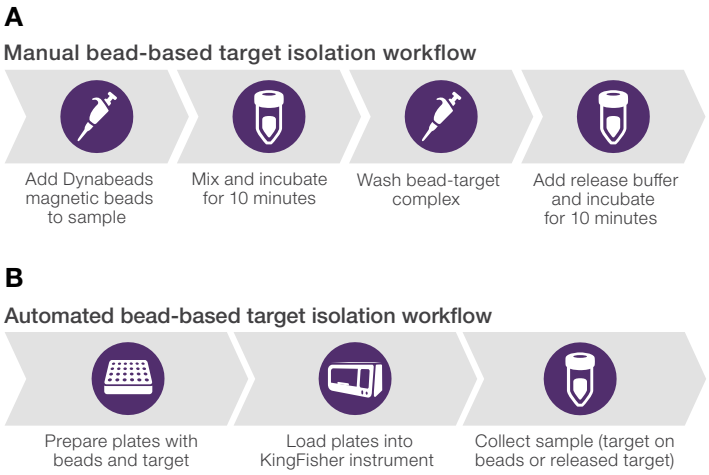

Figure 2. Exosome isolation workflow using a manual (A) or automated (B) method.

Results

Exosome size and concentration

For isolation and detection of exosomes, Dynabeads Intact Virus Enrichment beads were added to cell culture medium containing SW480 exosomes, followed by a 10-minute incubation. The isolation was performed manually or automated using the KingFisher Duo Prime instrument. The Spectradyne™ nCS1™ Particle Analyzer was used to measure the size and concentration of exosomes isolated and released from the Dynabeads Intact Virus Enrichment beads. Two cartridges were used to measure particle diameters in the ranges of ~65–400 nm (C-400) and ~250–2,000 nm (C-2000). A volume of 3 µL of exosomes diluted in a filtered solution of phosphate-buffered saline (PBS) with 1% Thermo Scientific™ Tween™ 20 detergent was added to the C-400 or C-2000 microfluidic cartridge. The exosome samples were analyzed in replicates.

High-quality size and concentration data from particles of diameters 65–400 nm (Figure 3A) and 250–2,000 nm (Figure 3B) were obtained. Sizes and concentrations of vesicles between 250 nm and 400 nm were measured by both cartridges and demonstrated good alignment (Figure 3C). Concentrations in replicate samples in the size range usually observed for exosomes were comparable (C-400). Both replicates demonstrated a detectable population of sizes above 1,000 nm that likely originated from the Dynabeads magnetic beads not being completely removed after the release (C-2000) (Figures 3B, 3C).

Exosome phenotype

The ARC Particle Analyzer was used to verify the identity of the vesicles. Exosomes isolated and released from Dynabeads Intact Virus Enrichment beads were stained with PE-labeled mouse anti–human CD81 antibody and incubated for 2 hours at room temperature in the dark. PE-labeled mouse IgG1 antibody was used as an isotype control. Prior to analysis, the labeled exosomes were further diluted 1:10 with 1X PBS containing 1% Tween 20 detergent. The single-particle fluorescence-based phenotyping (CD81 staining) showed a >10-fold higher PE-positive signal compared to the isotype control and indicates successful isolation of exosomes by the Dynabeads magnetic beads (Figure 3D).

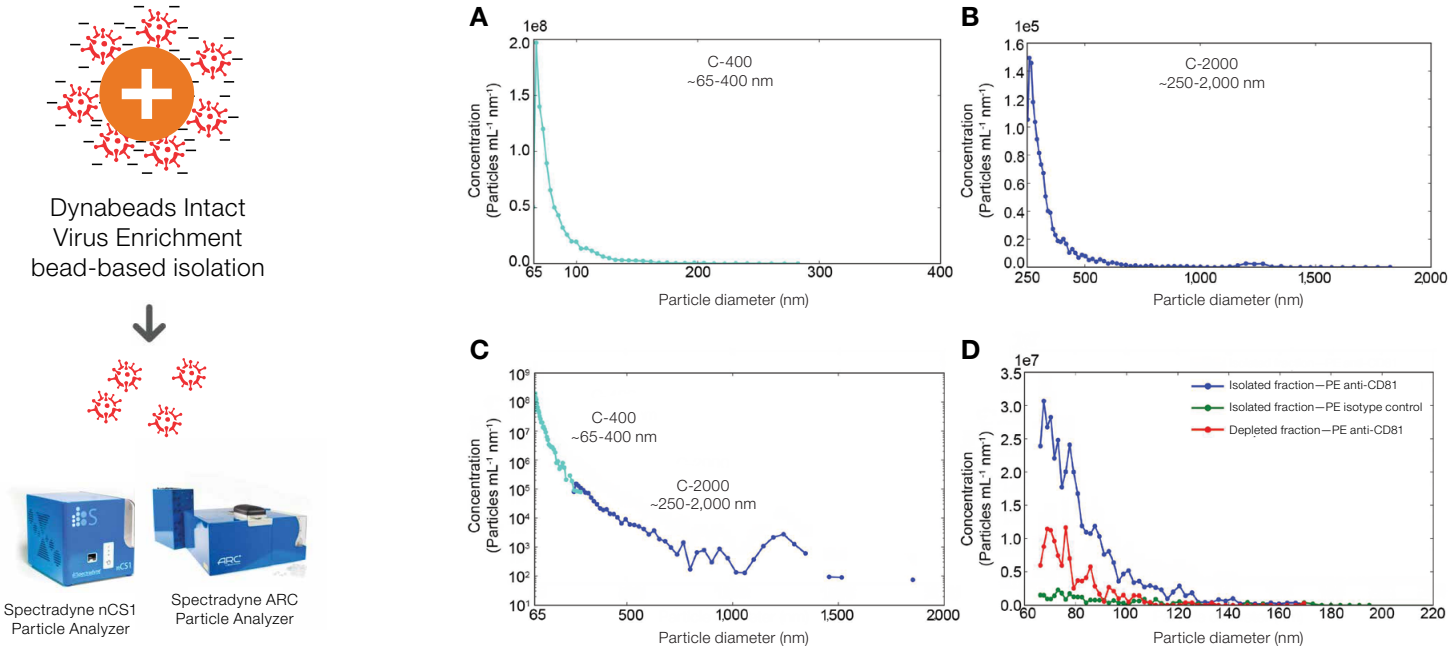

Figure 3. Multiomic analysis. Exosomes were isolated with the positively charged Dynabeads Intact Virus Enrichment beads, followed by release with 0.25 M KI in 20 mM triethanolamine. Size and concentration were measured with the Spectradyne nCS1 Particle Analyzer **(A–C)** according to the manufacturer's instructions. CD81-positive subpopulations of exosomes were quantified with the Spectradyne ARC Particle Analyzer according to the manufacturer's instructions. **(A)** High-quality size and concentration data from 65–400 nm exosomes. **(B)** High-quality size and concentration data from 250–2,000 nm exosomes. **(C)** Comparable size and concentration data across the 65–2,000 nm size range. **(D)** Size, concentration, and phenotyping of exosomes (CD81).

To further confirm the vesicle identity, the isolated exosomes were subjected to liquid chromatography–mass spectrometry (LC-MS). On-bead digestion with trypsin was performed prior to LC-MS analysis. The exosome markers CD9 and CD81 were detected at a probability of >95% (Figure 4).

These observations were further supported by western blot analysis. Exosomes isolated and released from Dynabeads Intact Virus Enrichment beads demonstrated the presence of the exosome marker CD81 in a western blot (Figure 5). Increasing the number of beads used for isolation increased the CD81 signal in the western blot (Figure 5A). Exosomes were efficiently released from the beads by adding 0.25 M KI in 20 mM triethanolamine to the bead-bound exosomes for 10 minutes at room temperature (Figure 5B).

Ultrastructural analysis of the isolated exosomes was performed by electron microscopy. The intact vesicles were observed to be surrounding the magnetic beads (Figure 6A). At higher magnification, the vesicular membranes of the structures were easily observed (Figure 6B). For identification, electron microscopy in combination with immunolabeling were used. After isolation, the vesicles were released from the beads and subjected to immunolabeling followed by negative-stain electron microscopy. The vesicles released were CD81-positive (Figure 6C), supporting the exosome nature of the isolated vesicles.

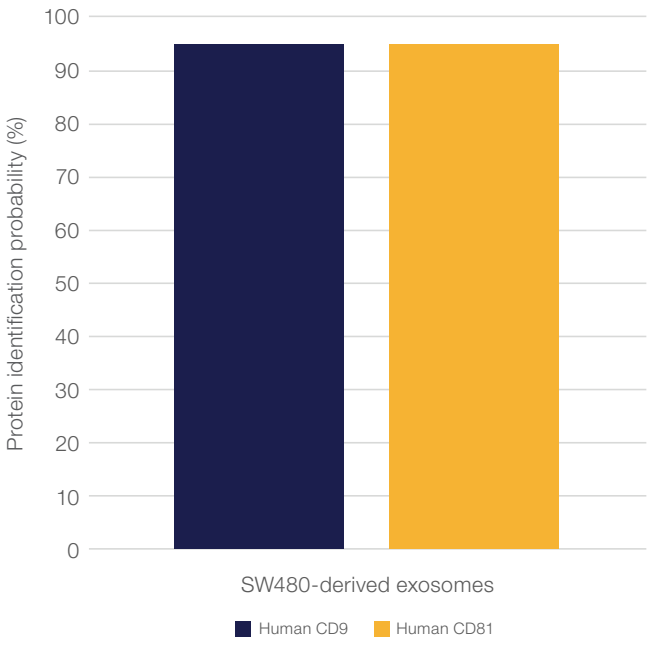

Figure 4. LC-MS analysis of exosomes isolated with Dynabeads Intact Virus Enrichment beads. CD9 and CD81 are exosome markers, which were detected at a probability of >95%.

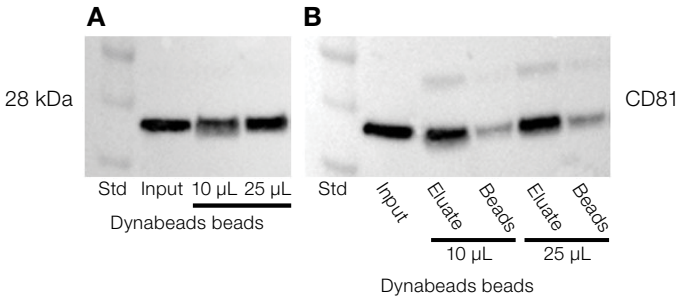

Figure 5. Isolation of exosomes with Dynabeads Intact Virus Enrichment beads. (A) Exosomes were isolated with 10 µL or 25 µL of beads and analyzed for CD81 by western blot. **(B)** Exosomes were isolated with 10 µL or 25 µL of beads, followed by release. The eluates and unreleased exosomes were analyzed for CD81 by western blot. Std = Invitrogen™ SeeBlue™ Plus2 Pre-stained Protein Standard.

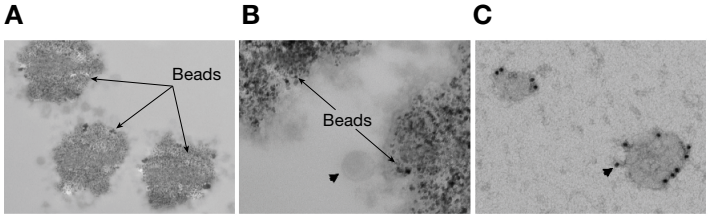

Figure 6. Ultrastructural analysis of exosomes isolated with Dynabeads Intact Virus Enrichment beads. Exosomes were isolated with strong anion exchange magnetic beads and processed for **(A, B)** transmission electron microscopy or **(C)** transmission electron microscopy in combination with immunolabeling and negative-stain analysis of released exosomes. **(A, B)** Exosomes on the surface of magnetic beads (arrowhead in **B** points to a vesicle structure). **(C)** Released exosomes labeled for CD81 (arrowhead points to a labeled vesicle).

Isolation efficiency

The isolation efficiency of the Dynabeads Intact Virus Enrichment beads was examined by flow cytometry (Figure 7A). Exosomes were isolated with 10 µL or 25 µL of the beads and then discarded. The remaining exosomes were isolated with Invitrogen™ Exosome-Human CD81 Flow Detection Reagent magnetic beads. Exosomes bound to the beads were stained with a PE-labeled anti-CD81 antibody and analyzed by flow cytometry. A gate was set around the single-bead population, and the mean fluorescence intensity (MFI) in the PE channel was recorded. Prior to depletion, the MFI was 2,363, and the same sample stained with the isotype control had an MFI of 140, which represents the background noise shown in gray (Figure 7B). Analysis of exosomes remaining after isolation using 10 µL or 25 µL of beads reduced the PE signal to 456 and 426, respectively (Figures 7C and 7D). This signal decrease demonstrates that the beads have depleted approximately 80% of the exosomes that were present in the samples.

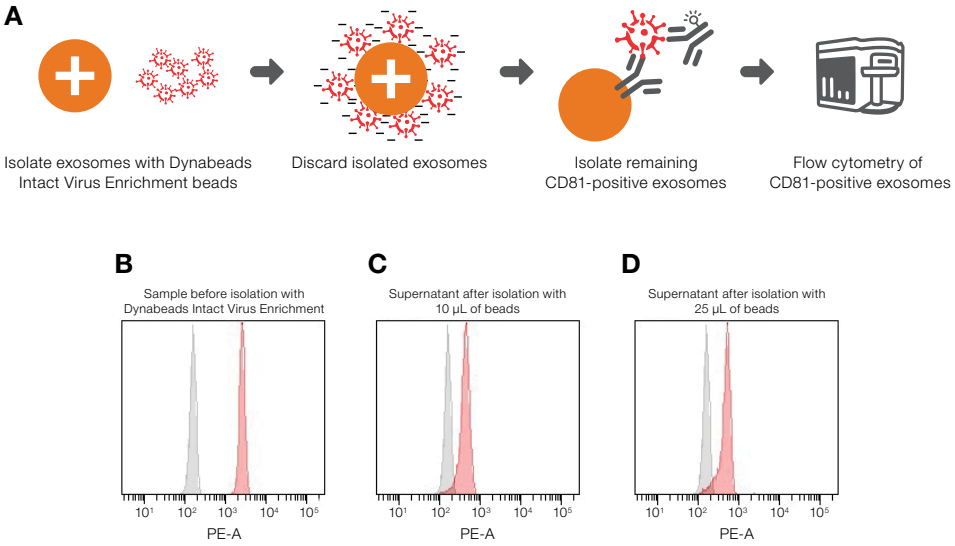

Figure 7. Flow cytometry analysis of isolation efficiency. (A) Exosomes were isolated from spiked samples (in PBS) with 10 µL or 25 µL of Dynabeads Intact Virus Enrichment beads. The isolated exosomes were then discarded. The remaining exosomes in the supernatant were isolated with Exosome-Human CD81 Flow Detection Reagent and stained with a PE-labeled anti-CD81 detection antibody, and finally analyzed by flow cytometry. **(B)** Exosomes present in the sample prior to isolation with Dynabeads Intact Virus Enrichment beads. Exosomes left in the sample after isolation of exosomes with **(C)** 10 µL or **(D)** 25 µL of beads. The gray histograms represent the isotype control **(B–D)**.

Recovery of released exosomes

Isolated and then released exosomes were recaptured using the Exosome-Human CD81 Flow Detection Reagent and processed for flow staining and analysis (Figure 8A). Exosomes isolated with 10 µL (Figure 8C) or 25 µL (Figure 8D) of Dynabeads Intact Virus Enrichment beads, released with 0.25 M KI, and recaptured with Exosome-Human CD81 Flow Detection Reagent demonstrated that the exosomes remained structurally undamaged throughout the whole process. For comparison, exosomes were captured directly with Exosome-Human CD81 Flow Detection Reagent and analyzed (Figure 8B). Each sample was compared to the isotype control using an irrelevant antibody of the same isotype (gray peaks).

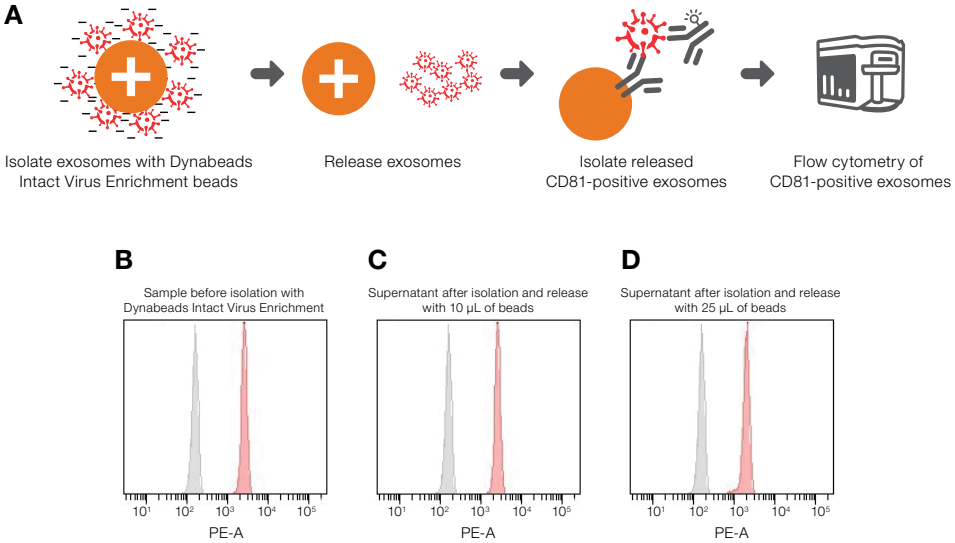

Figure 8. Flow cytometry analysis of exosomes released from Dynabeads Intact Virus Enrichment beads. (A) Exosomes were isolated from spiked samples (in PBS) with 10 µL or 25 µL of beads. The isolated exosomes were released from the beads with 0.25 M of KI and recaptured with Exosome-Human CD81 Flow Detection Reagent. The bead-bound exosomes were stained with PE-labeled anti-CD81 detection antibodies and finally analyzed by flow cytometry. **(B)** Exosomes present in the sample prior to Dynabeads magnetic beads isolation. Exosomes released after isolation with **(C)** 10 µL or **(D)** 25 µL of beads. Gray histograms represent the isotype control **(B–D)**. This demonstrates that the recovery of the released exosomes from the beads was high.

Isolation from serum

Finally, the isolation of exosomes from more biologically relevant material was tested. Exosomes derived from SW480 cells were spiked into serum, followed by isolation with the Dynabeads Intact Virus Enrichment beads. The exosomes were released from the magnetic beads with 0.25 M KI. The serum used was undiluted, or diluted to 10% and 50% in PBS. Released exosomes were analyzed on a western blot using an anti–human CD81 antibody (Figure 9A) or by recapture with Exosome-Human CD81 Flow Detection Reagent for downstream flow cytometry analysis (Figures 9B–9E). CD81 was detected in spiked and released serum samples, with serum diluted to 10% showing the highest band intensity (Figure 9A). No CD81 was observed after isolation and release from unspiked PBS or serum samples. CD81 was not detected on the Dynabeads Intact Virus Enrichment beads after release, suggesting that most of the exosomes were eluted off. Similar results were observed by flow cytometry. Exosomes captured and released from Dynabeads Intact Virus Enrichment beads were recaptured with Exosome-Human CD81 Flow Detection Reagent and analyzed by flow cytometry. Exosomes spiked into PBS or 10% serum demonstrated similar isolation and release efficiency (Figures 9B and 9C). Interestingly, less dilute serum samples (50% and 100% concentrations) resulted in slightly lower capture efficiency (Figures 9D and 9E). To investigate whether serum contains native CD81-positive exosomes, unspiked samples were included in the experiment. Blue peaks in the histograms represent the signals from unspiked samples and demonstrate that a small fraction of native CD81-positive exosomes are present in the serum. Each sample was compared to the isotype control using an irrelevant antibody of the same isotype (gray peaks).

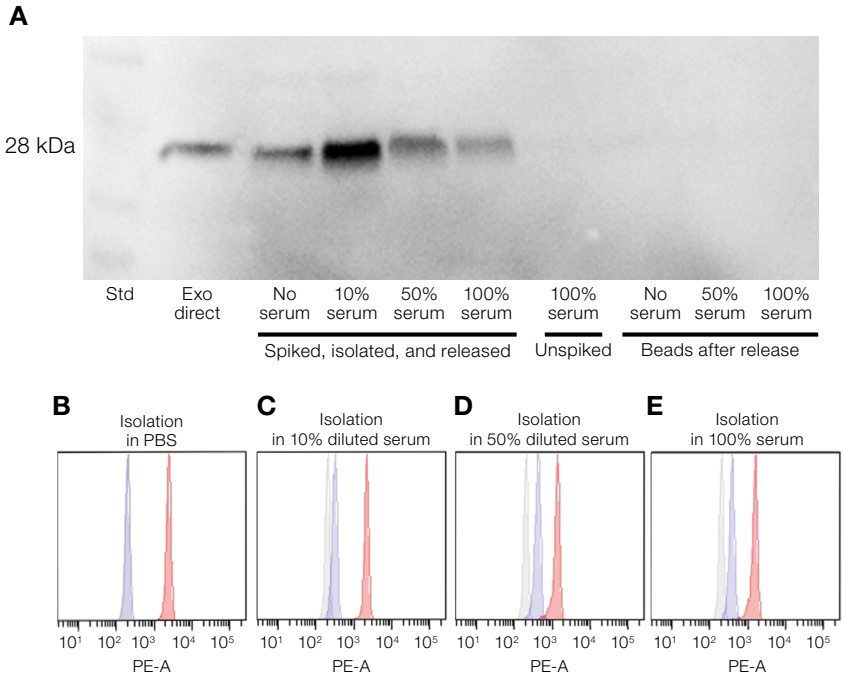

Figure 9. Isolation of exosomes from spiked and unspiked serum with Dynabeads Intact Virus Enrichment beads. (A) Exosomes were isolated from samples of 10%, 50%, or 100% serum in buffer (PBS). The isolated exosomes were released from the beads and analyzed for CD81 by western blot. For comparison, exosomes spiked into PBS, unspiked serum, and beads after release were included. **(B–E)** Flow cytometry analysis of exosomes released from the beads after isolation from PBS, or 10%, 50%, or 100% serum. Released exosomes were captured with Exosome-Human CD81 Flow Detection Reagent, stained for CD81 (PE-A), and analyzed by flow cytometry. Gray peaks represent the control isolation with an irrelevant antibody, blue peaks represent unspiked samples, and red peaks represent spiked samples.

Thermo Fisher SCIENTIFIC

Conclusions

Here we describe a simple, rapid, and reliable bead-based exosome isolation method based on the strong anion exchange (SAX) principle, using both manual and automated KingFisher protocols. Isolation was performed with Dynabeads Intact Virus Enrichment beads for multiomic analysis (size, concentration, and phenotype by Spectradyne particle analysis, western blot analysis, LC-MS analysis, and flow cytometry). We demonstrated successful isolation of intact exosomes spiked into PBS. In addition, both spiked and endogenous exosomes were isolated from serum samples using the same beads, and analyzed by western blot and flow cytometry. The same protocols can also be used to isolate and analyze negatively charged viruses such as SARS-CoV-2, adenoviruses, influenza viruses, noroviruses, Zika virus, Ebola virus, HIV, and respiratory syncytial virus (RSV), from virus transport medium or wastewater (data not shown). The automated protocol for rapid and efficient isolation of exosomes is compatible with the KingFisher Duo Prime, Flex, and Apex systems. The methods described here provide investigators with a simple, fast solution for rapid isolation of exosomes for multiomic exosome research.

Authors

Anette Kullmann, Berit Marie Reed, and Ketil Winther Pedersen, Thermo Fisher Scientific

Ordering information

Product name	Cat. No.
Dynabeads Intact Virus Enrichment	10700D
Exosome-Human CD81 Flow Detection Reagent	10622D
UltraPure Water	10977035
RIPA Lysis and Extraction Buffer	89900
4X Bolt LDS Sample Buffer	B0007
Bolt 4–12% Bis-Tris Plus Gel	NW04120BOX
20X Bolt MES SDS Running Buffer	B0002-02
SeeBlue Plus2 Pre-stained Protein Standard	LC5925
iBlot 2 Transfer Stacks, PVDF, mini	IB24002
CD81 Monoclonal Antibody (M38)	10630D
iBind Solution Kit	SLF1020
iBind Cards	SLF1010
SuperSignal West Dura Extended Duration Substrate	34076
KingFisher Duo Prime Purification System	5400110
KingFisher 12-Tip Comb	97003500
KingFisher Deep-Well Plate, v-bottom	95040450
Mini Gel Tank and Blot Module Set	NW2000
iBlot 2 Gel Transfer Device	IB2101
iBind Western Device	SLF1000
iBright FL1500 Imaging System	A44241
Attune NxT Flow Cytometer	A24860
Spectradyne nCS1 Particle Analyzer	nanoparticleanalyzer.com/index.php
Spectradyne ARC Particle Analyzer	
Spectradyne Microfluidic Cartridge C-400	
Spectradyne Microfluidic Cartridge C-2000	

Learn more at **thermofisher.com/exosomes**

invitrogen