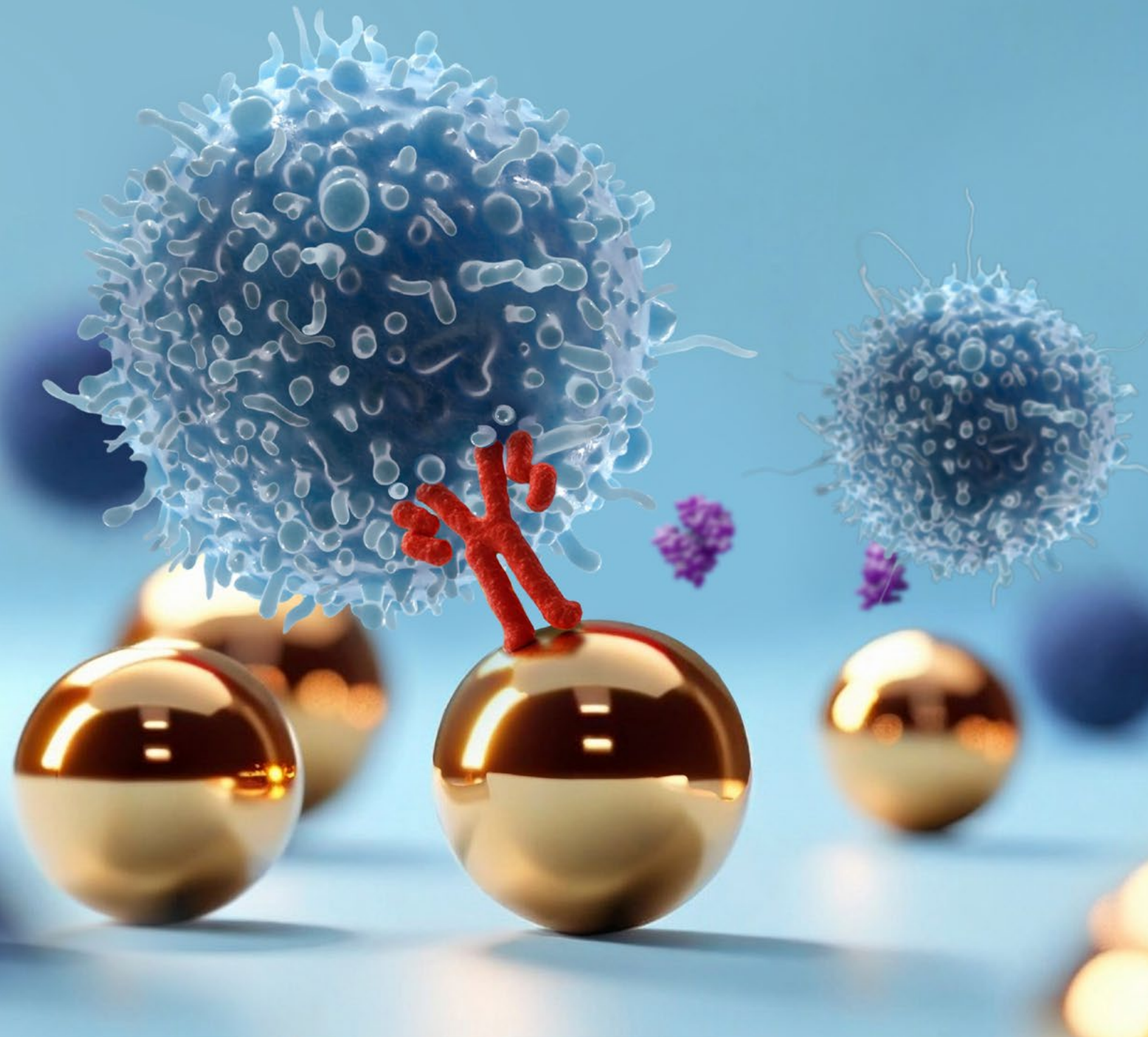
Cell analysis

Mastering cell isolation and exosome enrichment

Techniques and applications using magnetic bead technology



Contents

Cells

Isolation of cells with Invitrogen™ Dynabeads™ magnetic beads can be performed using three main techniques: positive isolation, negative isolation, cell depletion, or combinations thereof. Positive isolation can further be divided into positive isolation without bead release (resulting in bead-bound cells of interest) and positive isolation with bead release (resulting in bead-free cells of interest).

Dynabeads magnetic beads can be used directly in complex samples like whole blood, providing rapid access to target cells. They are typically precoated with target-specific antibodies (direct technique) or can bind to cells labeled with primary antibodies through secondary antibodies or streptavidin (indirect technique). Cell isolation using Dynabeads is fast, flexible, and requires no instrumentation other than a magnet and can be automated on platforms such as Thermo Scientific™KingFisher™ systems.

One of the unique properties of magnetic beads allows their use in various molecular studies, such as RT-qPCR or Western blot, without the need to remove the beads. However, for functional studies, beads should be detached. Invitrogen™ Dynabeads™ FlowComp™ products enable the removal of beads post isolation, and help ensure that no beads remain on the cells—an important factor for downstream applications like flow cytometry, cell culture, or differentiation assays. This clean separation is also critical for high-sensitivity techniques such as next-generation sequencing (NGS) and mass spectrometry, where residual beads can interfere with data quality and interpretation.

The efficiency of cell isolation using magnetic beads is influenced by factors such as incubation time, temperature, and reactant concentration. A critical step in achieving high performance is ensuring sufficient mixing of the beads with the cell sample, which maximizes contact between the beads and target cells. Key parameters, including the nature of the target cell, antigen-antibody binding characteristics, and the ratio of beads to cells, also play a significant role. High yield and purity are dependent on the optimal concentration of magnetic beads and the choice of antibodies, with monoclonal antibodies being preferred for their specificity.

Cell isolation methods

Dynabeads products enable gentle isolation of pure, viable, and functional target cells using either positive or negative isolation. Isolation of cells with Dynabeads magnetic beads can be performed using three main techniques: positive isolation, negative isolation, cell depletion, or combinations thereof.

- **Positive isolation without release:** Capture and isolate one cell type from the sample using beads that target a specific cell type of interest. Cells will remain bead bound.
- **Positive isolation with release:** Capture and isolate one cell type from the sample using beads that target a specific cell type of interest. The isolated cells are then released from the bead.
- **Negative isolation:** Enrich one cell population by using beads that target and remove all unwanted cell types.
- **Cell depletion:** Selectively capture and remove specific unwanted cells from the sample using beads that target that cell type.

Positive isolation generally helps provide higher purity, while negative isolation yields untouched cells, advantageous for applications where antibody binding might interfere with cell function.

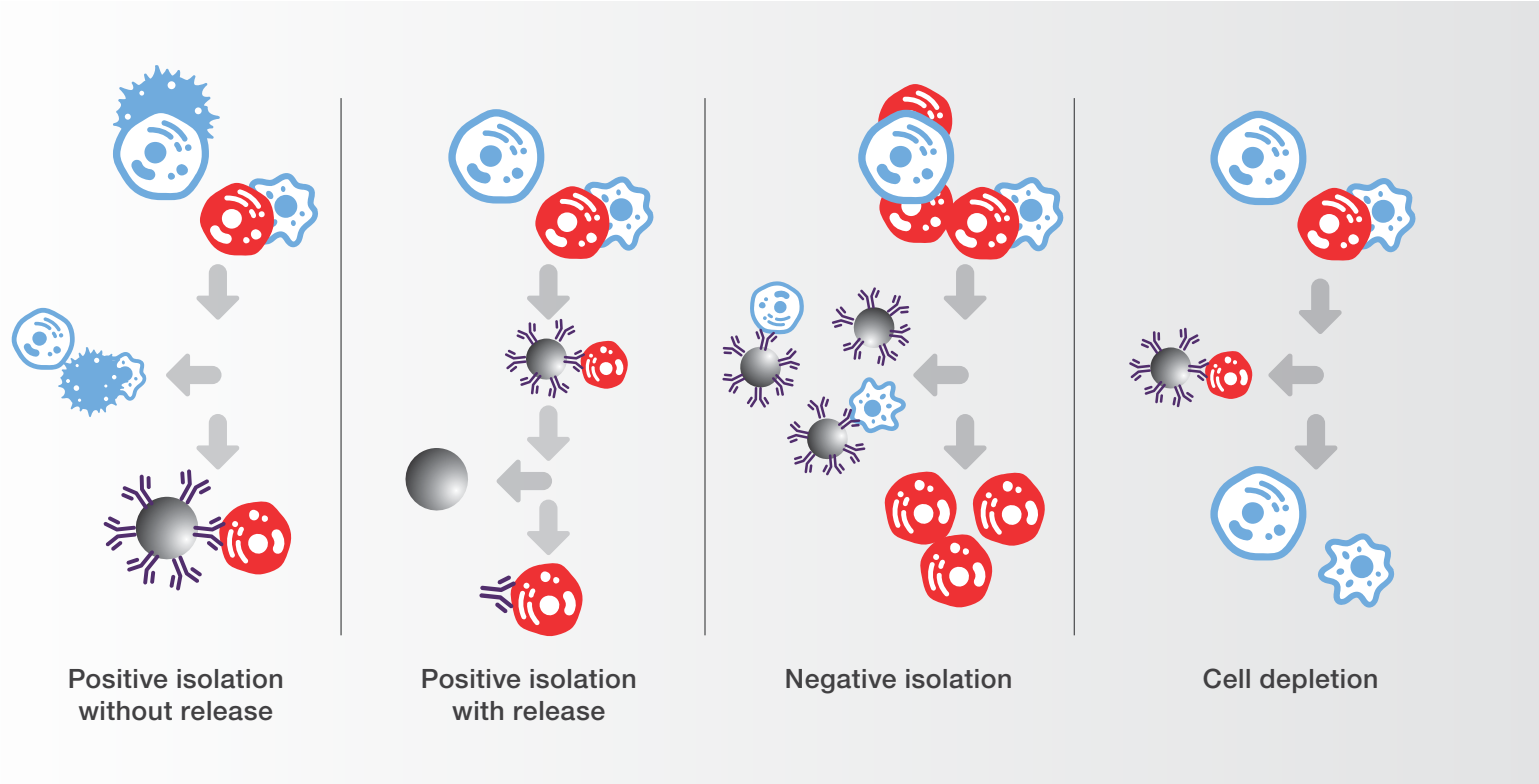


Figure 1. Overview of four cell isolation workflows covering positive and negative isolation.

Cell activation and expansion

T cells are very important cells in the immune system. These cells are part of the acquired immune response, responsible for fighting infections and many different diseases, including cancer. Understanding the mechanisms of naive T cell activation, maturation into effector and memory cells, re-activation of antigen-specific T cells and the function of regulatory T cells has been instrumental in the present developments of immune therapies including CAR-T cells (e.g. blood derived malignancies) and TCR cell therapies (e.g. solid tumors). Studies involving T cell activation and expansion have traditionally utilized antigen-presenting cells (APCs) to mimic *in vivo* activation processes. This method is labour-intensive and highly variable due to the need to prepare viable, functional APCs for each experiment.

Dynabeads magnetic beads for T cell activation offer an easy and reproducible method for activation and expansion of T cells *in vitro*, substituting the need for APCs (Figure 2). Dynabeads products, available for both human and mouse applications, enable bridging basic research and clinical use of T cells.

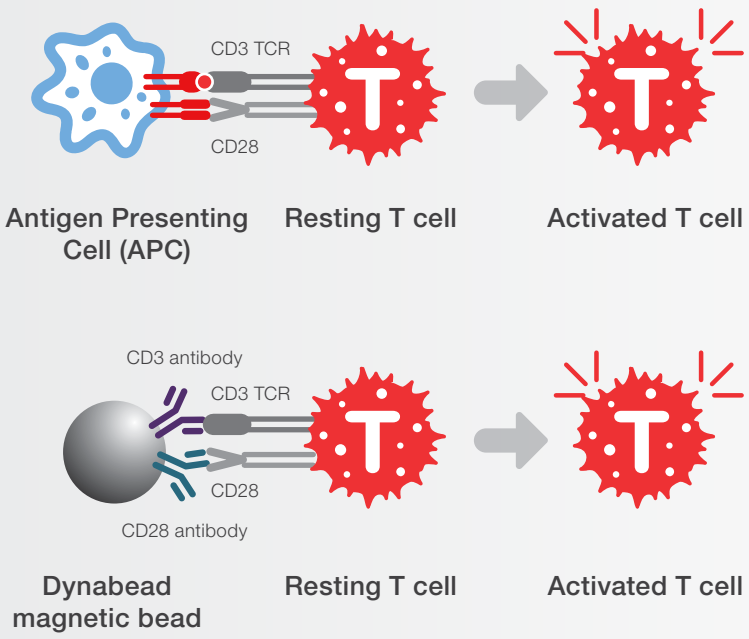


Figure 2. T cell activation by APC (*in vivo*) vs. T cell activation by Dynabeads (*in vitro*). (A) Resting T cells are activated for proliferation and differentiation by interaction with APCs, providing both primary and co-stimulatory signals via the T cell receptor/CD3 complex and CD28. (B) Dynabeads conjugated with antibodies against CD3 and CD28 are used *in vitro* to mimic the function of APCs to activate resting T cells.

Cell isolation

Positive isolation

Positive isolation of cells is performed using magnetic beads coated with cell surface marker–specific binders (generally specific antibodies). The isolated cell population is defined by expression of the surface markers targeted by the binders on the magnetic beads. Tow positive isolation workflows are available as described below.

Positive isolation without release

Positive isolation without bead release is an effective way to specifically target cells of interest but without the mechanism of releasing the cells. The sample is incubated with beads conjugated with a specific target antibody. The target cells are separated from the heterogenous population using a magnet. This method is suited for downstream molecular or proteomics applications as cells can be lysed directly on the beads.

Positive isolation with release

Positive isolation with release helps ensure a bead-free cell population for cell culture or flow cytometry applications. Invitrogen™ Dynabeads™ FlowComp™ Kits enable easy isolation of target cells with bead release. First, the sample is incubated with antibodies targeting the cells of interest. After a brief centrifugation to remove excess antibody, the targeted cells are captured by the FlowComp beads and separated from the unbound cells using a magnet. In a last step, the cells are released from the beads using a release buffer (Figure 3).

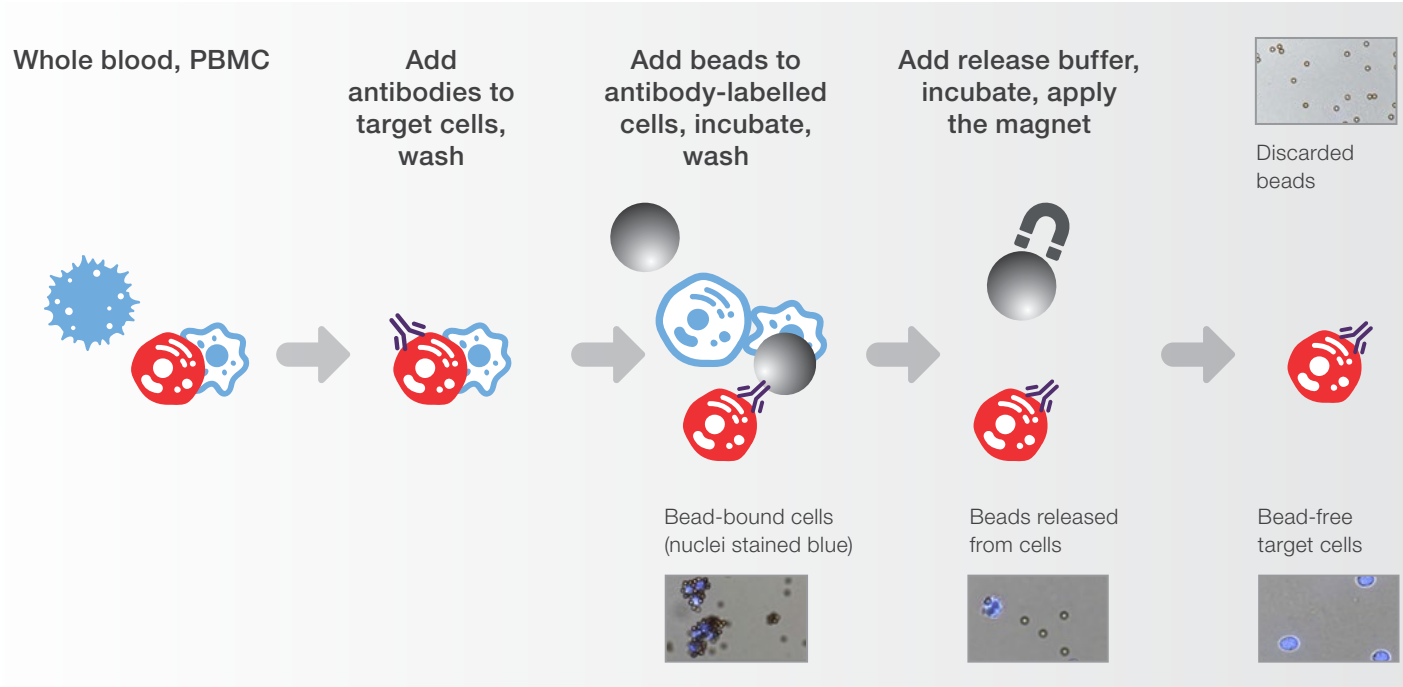


Figure 3. Positive isolation with release. A cell population is incubated with an antibody targeting cells of interest, followed by incubation with Dynabeads FlowComp magnetic beads. Captured cells are isolated using a magnet and released from the beads using release buffer.

Negative isolation

Negative isolation involves depleting unwanted cells from the sample. This method is recommended when the target cells shall remain untouched and free from any bead or antibody interference. The cells of interest are never bound to the antibodies on the magnetic beads, thereby avoiding potential antibody-induced signaling via cell surface molecules.

Invitrogen™ Dynabeads™ Untouched™ Kits are designed for efficient removal of unwanted cell types. Starting samples are typically human PBMCs and and tissue digests, such as mouse spleen and lymph nodes. An antibody mix specific to the unwanted cells is first added to the sample, followed by addition of magnetic beads that are specific for the labeling antibodies. The bead-cell complexes are removed using a magnet, leaving the pure and untouched cells of interest in the supernatant. Additional antibodies can be added to refine and increase specificity for a specific subpopulation. For customized negative isolation, samples can be incubated with any antibody mix of interest and subsequently depleted using secondary antibody-coated Dynabeads or Dynabeads streptavidin magnetic beads in combination with biotinylated antibodies.

Rare cell isolation

Download the application note

More details regarding CTC enrichment using positive or negative isolation can be found in this linked application note: Isolation of circulating tumor cells using Dynabeads magnetic beads.

Circulating tumor cells (CTCs) are increasingly recognized as valuable prognostic markers and tools for monitoring treatment response and thus plays a pivotal role in liquid biopsy research. However, isolating CTCs remains a significant challenge for scientists due to their low abundance in circulation and the requirement for highly sensitive methods to capture and detect them. To address these challenges, Dynabeads magnetic beads can be used both for positive and negative isolation of CTCs.

In positive isolation, Invitrogen™ Dynabeads™ Epithelial Enrich magnetic beads, coated with monoclonal antibodies specific for epithelial cell adhesion molecule (EpCAM), are used to capture CTCs directly from whole blood. Using positive isolation, down to one single target cell (e.g. CTC) can be efficiently isolated directly from 7.5 mL whole blood.

In negative isolation, CTC enrichment involves depleting CD45-positive leukocytes from blood-derived samples. Negative isolation offers the advantage of CTC-enrichment independent of antigen expression on the tumor cells. Using Invitrogen™ Dynabeads™ MyOne™ CD45 Leukocyte Depletion beads, all major lekocyte populations (lymphocytes, monocytes and granulocytes) are efficiently depleted from the sample.

Automated cell isolation

The cell isolation methods are both scalable and compatible with automation. Integrating an automated workflow for cell isolation will further simplify the cell isolation process (Figure 4). The Thermo Scientific™ KingFisher™ automated sample purification instrument scripts allow for high yield, purity, and viability, while reducing the cell isolation process to less than an hour for anywhere between 6 to 96 samples.

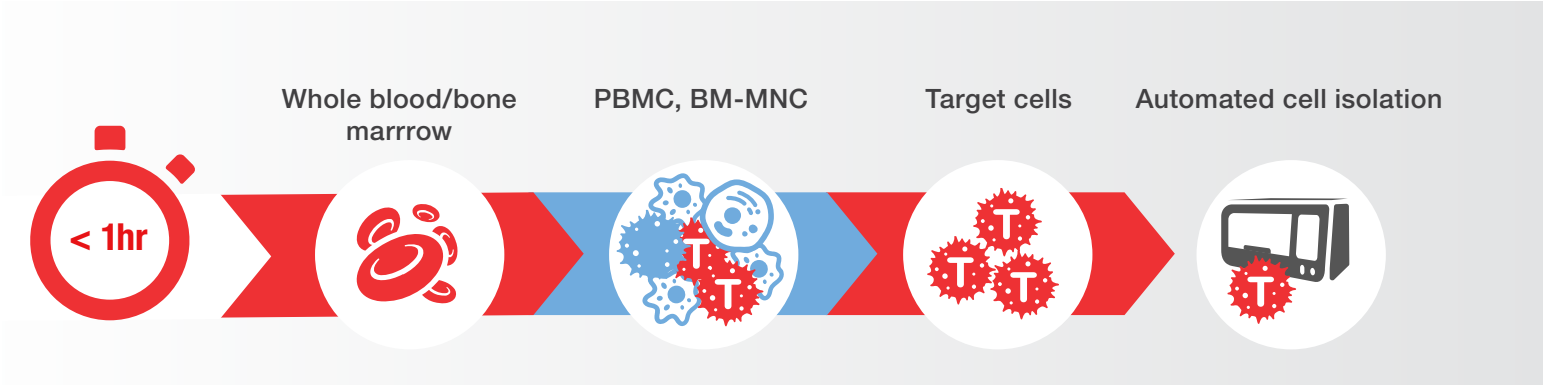
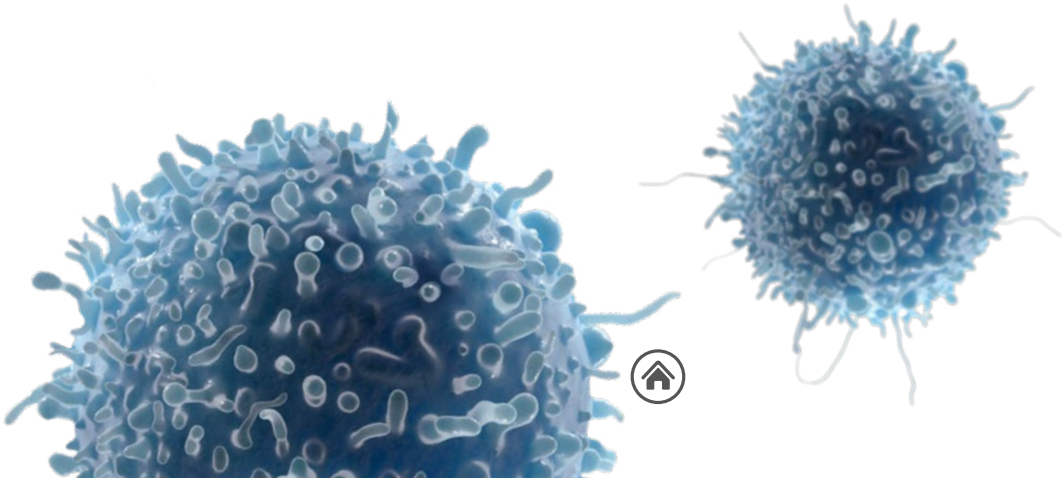


Figure 4. Automated cell isolation. Cell isolation can be achieved in less than an hour with KingFisher automation.

KingFisher scripts for cell isolation

KingFisher scripts are available for positive isolation for downstream applications such as T cell activation, utilizing Dynabeads FlowComp Kits including CD3, CD4, and CD8. Additionally, scripts are available for positive isolation for downstream nucleic acid or protein analysis, or cell depletion, utilizing Dynabeads specific for cells including CD3, CD4, CD8, CD14, CD15, CD19 Pan B, Epithelial Enrich, and CD45.

Learn more about KingFisher instruments at thermofisher.com/kingfisher



Cell depletion

Cell depletion is a highly effective method for refining cell populations by selectively removing unwanted cells from a heterogeneous sample. Magnetic beads conjugated with antibodies targeting one specific cell marker bind to the unwanted cells that are then removed applying a magnet. The desired cell populations in the supernatant are free of antibodies and beads. Figure 5 shows CD3-positive cell depletion as an example. Target cell depletion efficiency was above 99% as determined by flow cytometry.

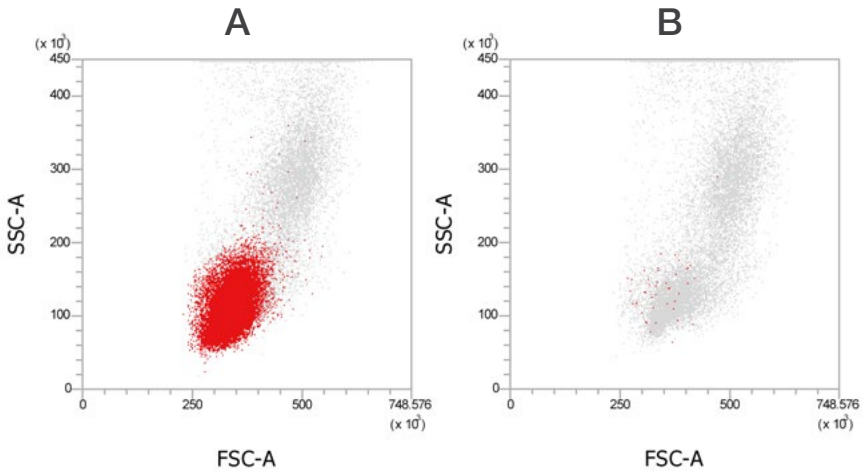


Figure 5. Cell depletion of CD3-positive cells from PBMC using automation. Invitrogen™ Dynabeads™ CD3 magnetic beads were used to deplete CD3-positive T cells from PBMC using Thermo Scientific KingFisher instrument automation. Flow analysis: scatter plots show CD3-positive T cells in red before (A) and after (B) cell depletion.

T cell activation and expansion

Understanding the various functions of immune cells is of great importance and requires both adequate isolation and activation/expansion techniques. Dynabeads magnetic beads address the need for cell isolation, activation and expansion of both human and mouse T cells.

Dynabeads conjugated with antibodies against CD3 and CD28 offer an easy and reproducible method for activation and expansion of T cells *in vitro*, substituting the need for APCs (Figure 6). The beads work by providing a fine-tuned balance between signal 1 (via the T cell receptor/CD3 complex) and signal 2 (co-stimulation via CD28) to activate T cells.

- **Gibco™ Dynabeads Human T-Activator CD3/CD28 beads:** designed for T cell activation and expansion studies starting with naïve human T cells or CD4- and CD8-positive T cell subsets
- **Gibco™ Dynabeads Human T-Activator CD3/CD28/CD137 beads:** designed for T cell activation and expansion studies starting with antigen specific T cells, as these cells benefit from co-stimulation through the CD137 receptor promoting both survival and proliferation of the T cells
- **Gibco™ Dynabeads Human Treg Expander beads:** designed for activation and expansion studies starting with regulatory T cells (CD4/CD25 positive T cells)

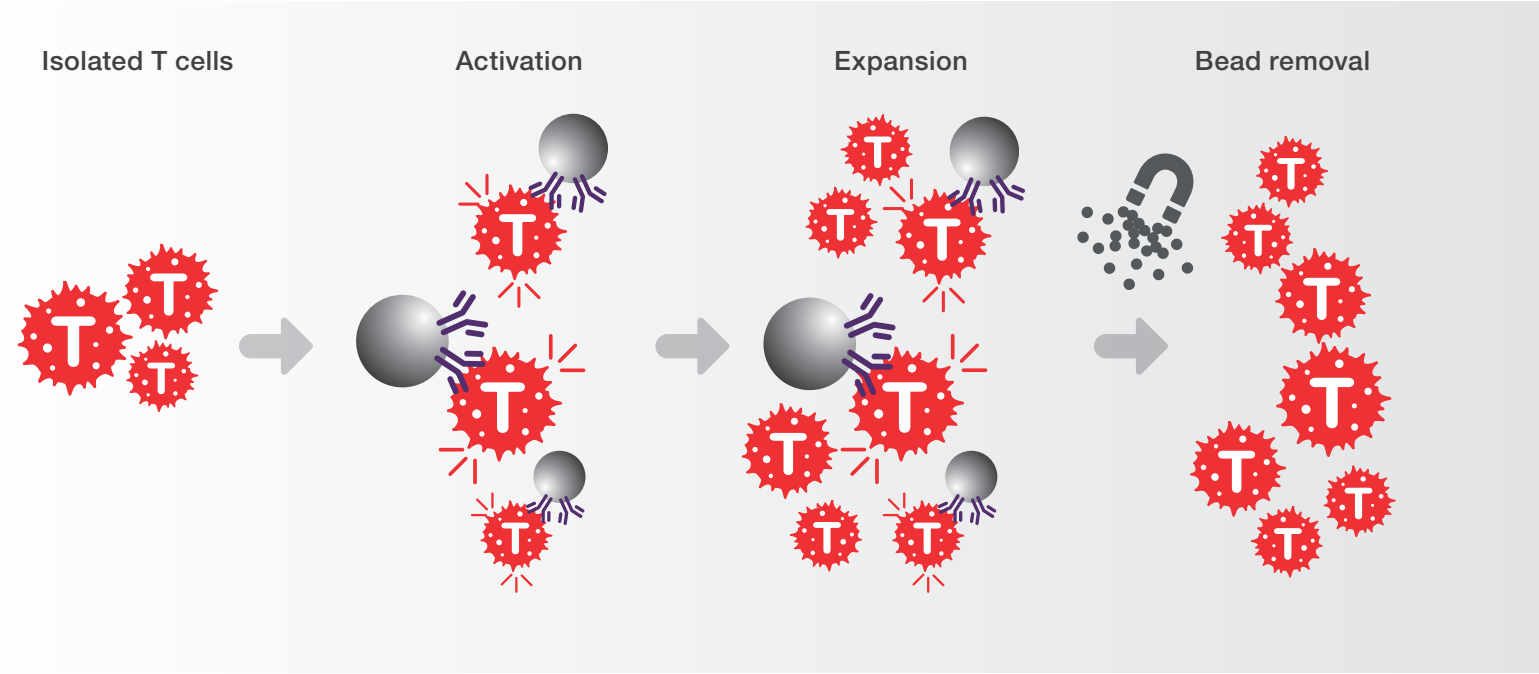


Figure 6. T cell isolation and expansion. T cells are first isolated using positive or negative isolation. The cells are then incubated with Dynabeads Human T-Activator CD3/CD28 beads. Upon activation and 2-3 days thereafter, T cells stop binding to the beads due to recycling/down-regulation of the cell surface receptors. When detached, the beads can be removed using a magnet.

Automated T cell isolation for downstream activation

Download the application note

More details regarding automated cell isolation and immuno-precipitation can be found in: Automated T cell isolation featuring easy integration with downstream activation and immunoprecipitation.

The first step of T cell activation is cell isolation. Cells can be isolated by either positive or negative isolation, and the workflow can be automated on KingFisher systems. T cell activation and proliferation are consistent and robust and are not affected by cell isolation methods. While T cell purity is slightly higher applying positive isolation compared to negative isolation, there are no differences in downstream cell activation.

KingFisher automation can be used for isolation of proteins, cells, and nucleic acids from a variety of sample types. For example, its automation capabilities can streamline a cell isolation, T cell activation, and immunoprecipitation workflow, reducing the workflow time from traditional 2-3 days to approximately 7 hours (Figure 7). Moreover, automated immunoprecipetation (IP) and subsequent Western blot processing minimize manual errors, increase throughput, and ensure reproducibility. The integration of automated systems into cell isolation workflows marks a pivotal step towards more efficient and operator independent cellular analysis.

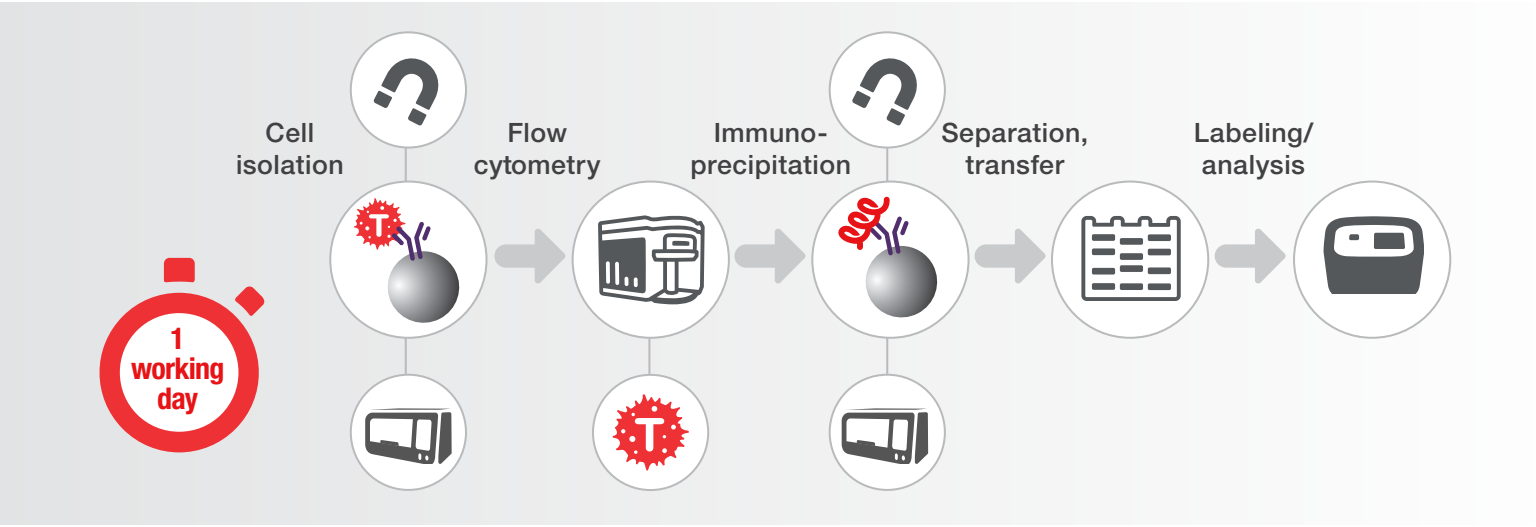


Figure 7. Automated cell isolation. Cell isolation and downstream applications are achievable within one working day.

B cell activation using activated T cells

Download the application note

More details regarding B cell activation can be found in: Stimulation and expansion of human B cells by magnetic bead-based activation of CD4+ T cells.

T cells activated using Dynabeads Human T-Activator CD3/CD28 magnetic beads can be used to activate B cells into plasma or memory B cells. Activated CD4-positive T helper cells are activated to express the necessary receptors that specifically promote B cell activation. This approach can be adopted for studying antibody production and a range of B cell malignancies.

Cell isolation and activation products

Products for cell isolation for human cells, mouse cells, and products for T cell activation are listed in the following tables.

Table 1. Positive isolation products (human cells)

Product	Cat. No.
Positive isolation with release	
Dynabeads products are optimized for various cell isolation techniques. Dynabeads FlowComp Kits enable positive isolation with a release mechanism. This process enables the user to obtain a pure bead-free target cell population.	
Dynabeads CD4 Positive Isolation Kit	11331D
Dynabeads CD8 Positive Isolation Kit	11333D
Dynabeads CD34 Positive Isolation Kit	11301D
Dynabeads FlowComp Human CD3	11365D
Dynabeads FlowComp Human CD4	11361D
Dynabeads FlowComp Human CD8	11362D
Dynabeads FlowComp Human CD14	11367D
Dynabeads Regulatory CD4+CD25+ T Cell Kit	11363D
DETACHaBEAD CD19 Kit	12506D
Positive isolation without release and cell depletion	
Dynabeads targeting multiple CD markers without a release mechanism are available for both positive isolation without release and depletion.	
Dynabeads CD2	11159D
Dynabeads CD3	11152D
	11151D
Dynabeads CD4	11146D
	11145D
Dynabeads CD8	11148D
	11147D
Dynabeads CD14	11150D
	11149D
Dynabeads CD15	11138D
	11137D
Dynabeads CD19 PAN B	11144D
	11143D
Dynabeads CD25	11157D
Dynabeads CD45	11153D
Dynabeads CD31 Endothelial Cell	11155D
Dynabeads MyOne CD45	11170D
	11171D
Dynabeads Epithelial Enrich	16103D
	16102

Table 2. Negative isolation products (human cells)

Product	Cat. No.
Negative isolation	
For negative isolation, Invitrogen™ Dynabeads™ Untouched™ Kits cater to a wide range of target cells. Additionally, cell isolation can be customized using specific antibodies in combination with secondary-coated Dynabeads such as Invitrogen™ Dynabeads Biotin Binder and Invitrogen™ Dynabeads™ Pan Mouse IgG.	
Dynabeads Untouched Human T Cells	11344D
Dynabeads Untouched Human CD4 T Cells	11346D
Dynabeads Untouched Human CD8 T Cells	11348D
Dynabeads Untouched Human NK Cells	11349D
Dynabeads Untouched Human Monocytes	11350D
Dynabeads Untouched Human B Cells	11351D
Secondary Coated Beads	
Dynabeads Biotin Binder	11048D
	11047
CELLection Biotin Binder	11533D
Dynabeads Pan Mouse IgG	11040D
	11041
	11042
Dynabeads Sheep Anti-Mouse IgG	11031
Dynabeads Sheep Anti-Rat IgG	11035
	11036D
Dynabeads FlowComp Flexi Kit	11061D

Table 3. T cell activation/expansion products

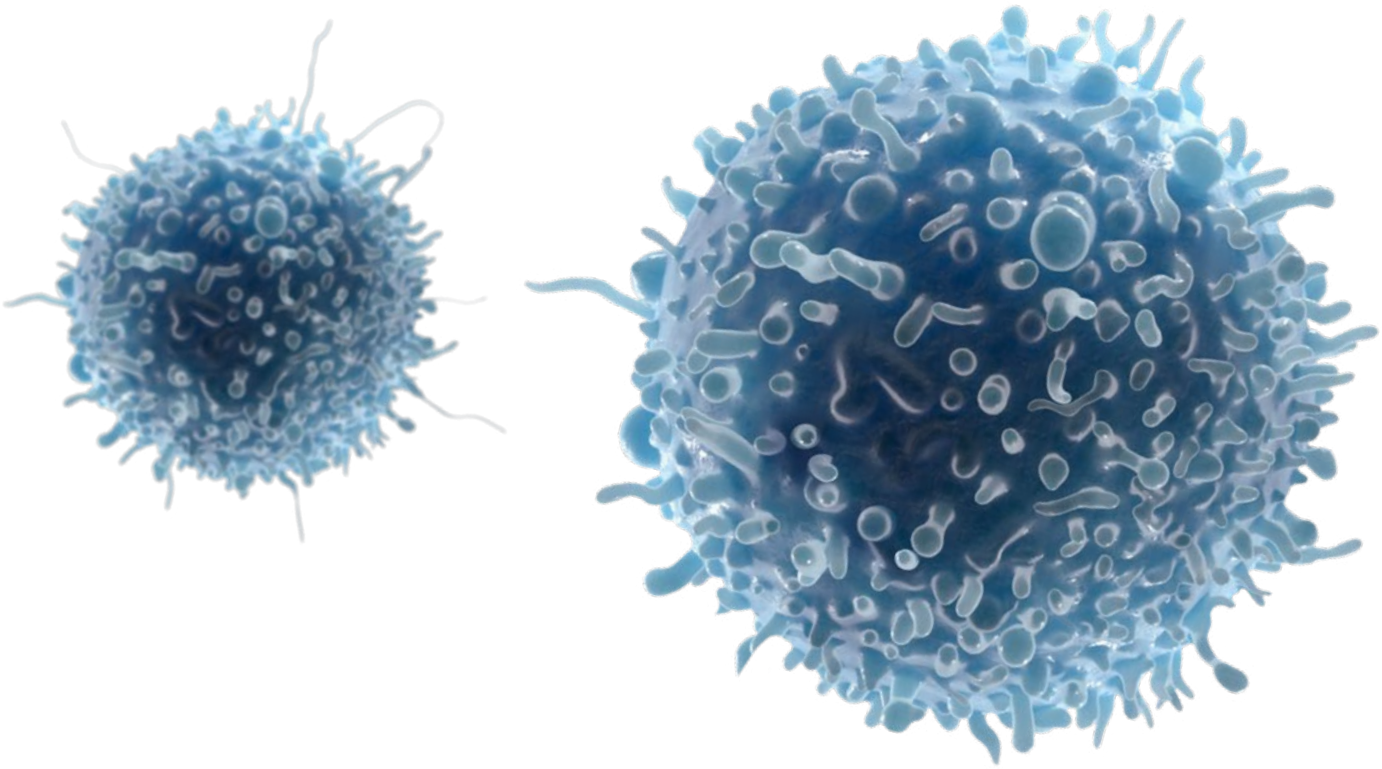
Product	Cat. No.
Human	
Dynabeads Human Treg Expander	11129D
Dynabeads Human T-Activator CD3/CD28	11131D
	11132D
	11161D
Dynabeads Human T-Activator CD3/CD28/CD137	11162D
	11163D
Mouse	
Dynabeads Mouse T-Activator CD3/CD28	11452D
	11453D
	11456D

Cell isolation and activation product overview, continued

Table 4. Isolation products (mouse cells)

Product	Cat. No.
Positive Isolation with release Dynabeads products are optimized for various cell isolation techniques. Dynabeads FlowComp Kits enable positive isolation with a release mechanism. This process enables the user to obtain a pure bead-free target cell population.	
Dynabeads FlowComp Mouse CD4	11461D
Dynabeads FlowComp Mouse CD8	11462D
Dynabeads FlowComp Mouse PAN T	11465D
Positive isolation without release and cell depletion Dynabeads targeting multiple CD markers without a release mechanism are available for both positive isolation without release and depletion.	
Dynabeads Mouse CD4 (L3T4)	11445D
Dynabeads Mouse CD8 (LYT2)	11447D
Dynabeads Mouse Pan B (B220)	11441D
Dynabeads Mouse PAN T (THY1.2)	11443D

For more information on T cell expansion, please visit our website thermofisher.com/cellactivation



Exosomes

What are extracellular vesicles and exosomes

Extracellular vesicles (EVs) are membrane-bound particles released by cells, including exosomes, microvesicles, and apoptotic bodies, each with distinct biogenesis pathways and functions. EVs have gained research interest for their role in biomarker and liquid biopsy assays, often considered superior to circulating free DNA (cfDNA) and circulating tumor cells (CTCs). Among EVs, exosomes (30-150 nm) are particularly significant because they carry various molecules, including nucleic acids, proteins, and lipids.

Exosomes, secreted by all cells and found in all body fluids, are formed by the invagination of the endosomal membrane to create multivesicular bodies (MVBs). These MVBs fuse with the plasma membrane, releasing exosomes into the extracellular environment. Exosome cargo includes DNA, miRNA, mRNA, tetraspanins (e.g., CD9, CD81), heat shock proteins, lipid-related proteins, lipids, and metabolites. Exosomes mediate intracellular communication over short and long distances by transporting their cargo, which reflects their cell of origin. They play crucial roles in several biological processes, including facilitating the immune response, enabling apoptosis, promoting angiogenesis, inflammation, blood coagulation, and the metastasis and progression of tumors. Tumor-derived exosomes can suppress immune responses, transfer oncogenes, and affect intercellular communication, although the exact mechanisms are still under investigation.

Exosome enrichment

There are currently no standard approaches for exosome enrichment or separation. Methods typically follow three different primary concepts. The approaches aim to (1) “concentrate” by reducing volume while retaining the target vesicles together with other materials, (2) “enrich” by retaining the vesicles while reducing the number of unwanted materials, or (3) “separate” by utilizing the combination of certain biophysical and/or biochemical properties of the exosomes. While the “concentrate” approach has high efficiency but low specificity, the “enrich” approach provides medium efficiency and specificity, and the “separate” approach provides low efficiency but high specificity.”

Identification of the enriched and separated exosomes often relies on a combination of methods, including electron microscopy for ultrastructural analysis by size, and concentration distribution measurements by, for example, resistive pulse sensing method or light scattering–based methods (dynamic light scattering and nanoparticle tracking analysis) in combination with flow cytometry, RT-qPCR, and western blotting.

Generic exosome enrichment

Traditional exosome enrichment methods include differential ultracentrifugation, density gradients, size exclusion chromatography, and precipitation. While these approaches are widely used, they present challenges such as low yield, contamination, high cost, time-consuming protocols, scalability issues, and inconsistent reproducibility. Among these methods, ultracentrifugation has been the standard for isolating exosomes from cell culture media and body fluids. Although it is valued for recovering clean exosomes, it has significant limitations. These include the need for extensive training, large sample volumes, low yields, and labor-intensive, time-consuming procedures. In addition, the process lacks scalability, limiting throughput to a maximum of six samples at a time, and results can be inconsistent, with exosome fractions or pellets easily lost during handling.

To address the need for scalable, simplified, and time-efficient generic exosome enrichment, we offer two advanced solutions: precipitation-based enrichment using Invitrogen™ Total Exosome Isolation Reagent (Figure 1A), optimized for different starting samples, and charge-based magnetic enrichment using Dynabeads magnetic beads (Figure 1B). These methods provide reliable alternatives to traditional techniques, increasing efficiency and adaptability to different research needs.

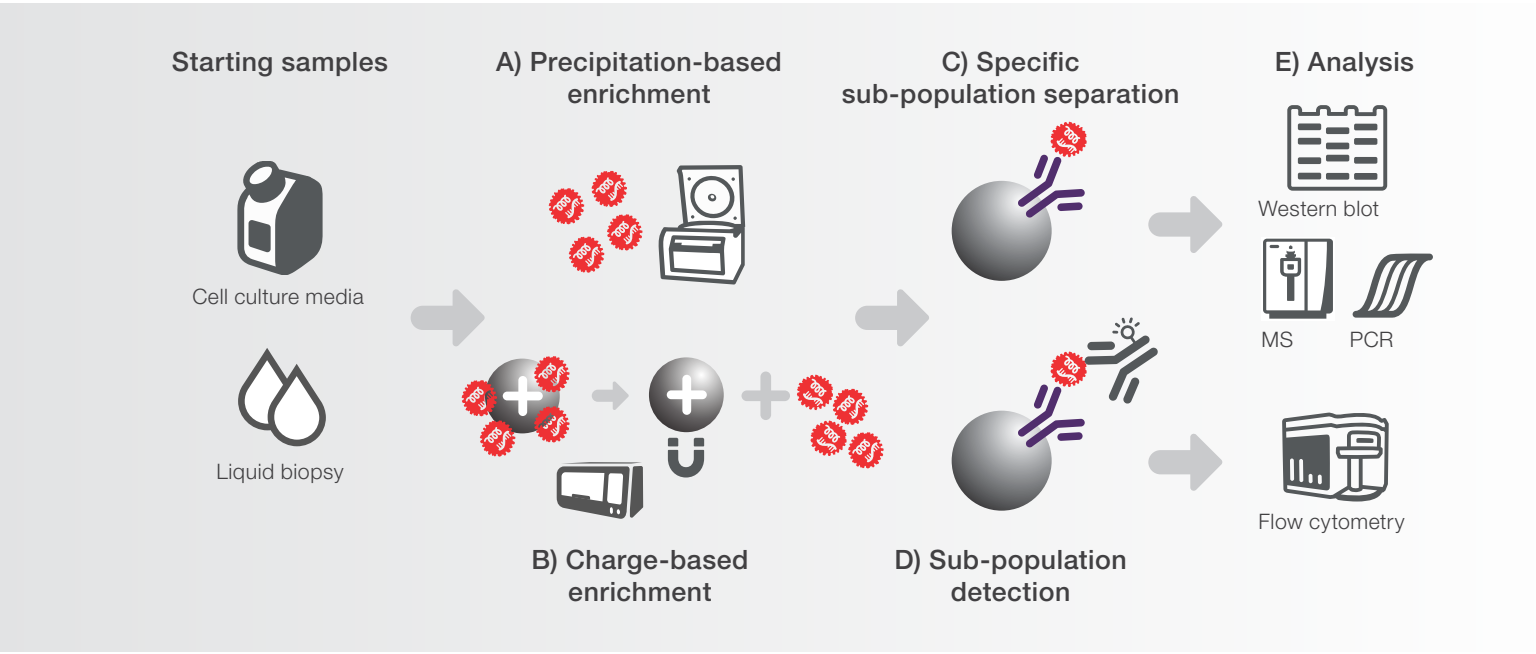


Figure 1. Exosome enrichment, separation and detection. (A) Precipitation with Total Exosome Isolation Reagent Kits (specific for cell culture media, urine, plasma, and other samples). (B) Charge-based enrichment with Invitrogen™ Dynabeads Intact Virus Enrichment magnetic beads with release. (C) Sub-population separation with Dynabeads magnetic beads. (D) Sub-population detection with Dynabeads magnetic beads for flow analysis. (E) Downstream analysis.

Total Exosome Isolation Reagents provide a simple and reliable method for the enrichment of intact exosomes from cell culture media and various body fluids, including serum, plasma, urine, saliva, cerebrospinal fluid, milk, ascitic fluid, and amniotic fluid. These reagents work by binding water molecules, forcing less soluble components—such as exosomes—out of the solution. The exosomes can then be easily collected after a brief, low-speed centrifugation, streamlining the traditionally labor-intensive enrichment process.

Charge-based magnetic enrichment using Invitrogen™ Dynabeads Intact Virus Enrichment magnetic beads provides a streamlined and efficient exosome enrichment process suitable for a variety of starting materials, including cell culture media and liquid biopsy samples such as serum, plasma, and urine. Dynabeads Intact Virus Enrichment magnetic beads are strong anion exchange magnetic beads that are positively charged throughout the pH range. The negatively charged exosomes quickly replace the surrounding negatively charged Cl^- anions due to their higher relative affinity, leading to very rapid binding within minutes (Figure 2). This reversible process uses a release solution selected based on ion affinity as a function of charge and hydrated size, following the order of relative affinity of anions on strong base anion exchangers. This makes the enrichment method fast, reversible, and suitable for automation (Figure 3). The released exosomes maintain their integrity.

Benefits of using Dynabeads Intact Virus Enrichment beads are:

- **Speed:** Intact exosome enrichment in ~20 minutes
- **Simplicity:** Rapid protocol that can be automated
- **High throughput:** Enrich up to 96 samples per run
- **Functionality:** Intact exosomes can be used in various functional assays
- **Quick release:** Release exosomes from the beads in 10 min

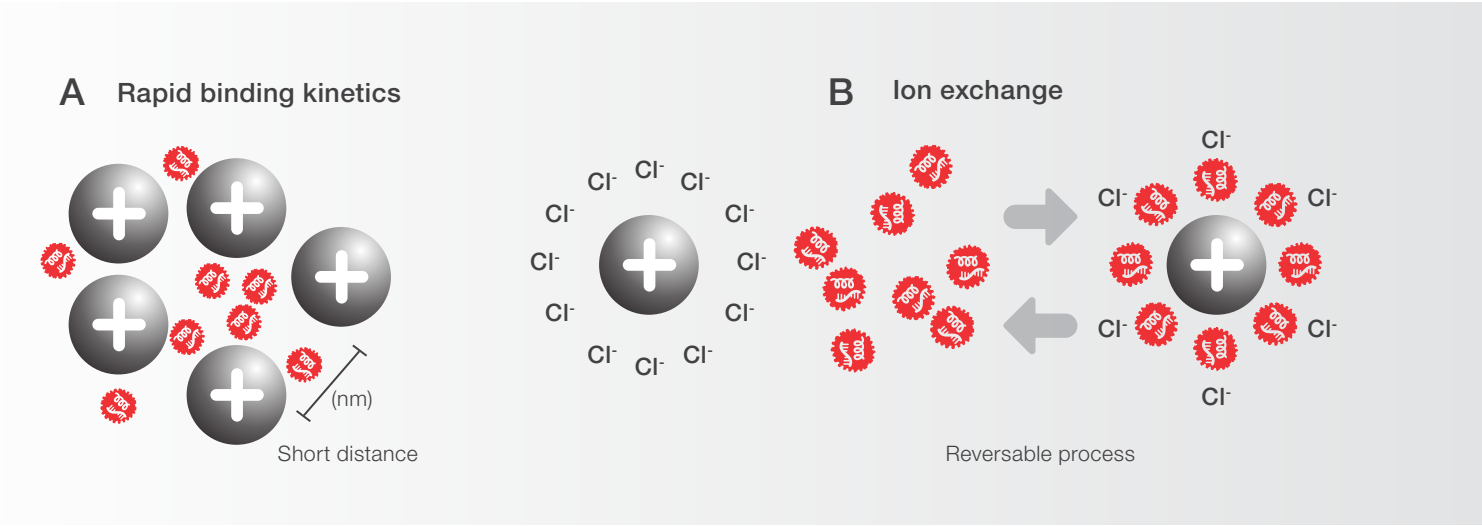


Figure 2. Charge-based magnetic enrichment principle. (A) The positively charged Dynabeads Intact Virus Enrichment beads are near the negatively charged exosomes, enabling rapid binding kinetics and a fast enrichment protocol. (B) Positively charged Dynabeads Intact Virus Enrichment beads with Cl^- ions are used for the generic enrichment of negatively charged exosomes. 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.

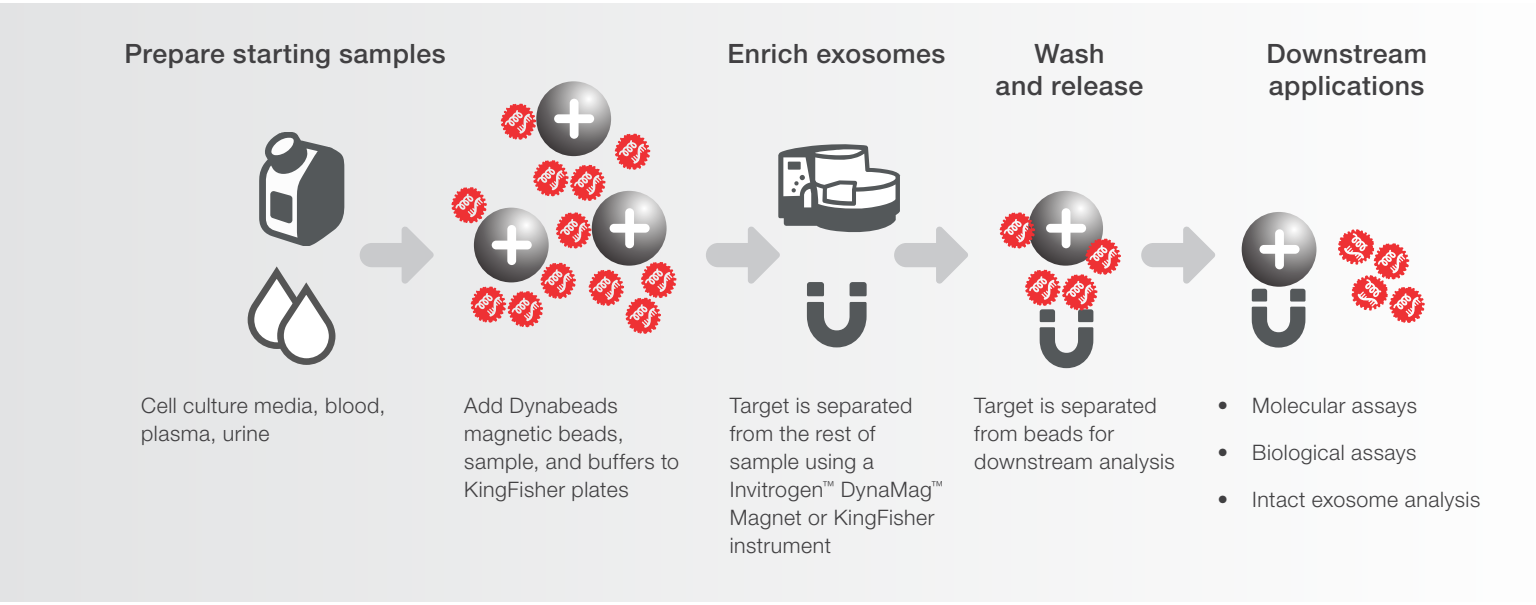


Figure 3. Sample exosome enrichment workflow using Dynabeads Intact Virus Enrichment is automated on the KingFisher instrument.

Specific exosome separation

To separate exosome subpopulations, a combination of biophysical and biochemical characteristics is used. Initially, strong anion exchange beads can be applied for rapid enrichment, which takes about 10–20 minutes. After this, specific exosome subpopulations can be isolated by targeting classic exosome surface proteins (CD9, CD63, and CD81) using Dynabeads magnetic separation technology (Figures 1C and 1D). Exosomes are captured with either Dynabeads conjugated with anti-CD9, Dynabeads conjugated with anti-CD63, or Dynabeads conjugated with anti-CD81 antibodies, following an overnight incubation. For other exosomal targets, Invitrogen™ DynaGreen™ magnetic beads used in combination with a primary antibody enable separation within 40 minutes.

Invitrogen™ Exosome-Human Isolation Kits utilize Dynabeads magnetic beads pre-conjugated with monoclonal anti-CD9, anti-CD63, or anti-CD81 antibodies to purify exosome subpopulations from cell culture media. These purified exosomes can be analyzed using techniques such as Western blotting or quantitative PCR (qPCR). For applications such as flow cytometry or electron microscopy, where magnetic beads are used as a solid support and exosome carriers, Invitrogen™ Exosome-Human CD9 Flow Detection reagents, for anti-CD9, anti-CD63, or anti-CD81 antibodies should be used. The monodisperse Dynabeads provide a distinct forward and side scatter (FSC/SSC) signal, allowing for rapid analysis, typically in under one hour.

Key advantages of using Dynabeads for separating exosome surface proteins (CD9, CD63, and CD81) include:

- **Efficiency:** Minimal hands-on time for efficient exosome subset separation
- **Purity:** High purity of the separated exosome subset
- **Flexibility:** Scalable protocol to suit various sample sizes
- **Functionality:** Compatibility with most downstream analysis methods

Download the application note

To learn more about magnetic bead-based workflows in multiomic research, please read our application note: Rapid bead-based isolation of exosomes for multiomic research.

Invitrogen™ DynaGreen™ CaptureSelect™ Anti-IgG-Fc magnetic beads (multi-species) are microplastic-free submicron magnetic beads with a low sedimentation rate, allowing efficient separation of exosomes in 40 minutes. The beads are covalently conjugated with a recombinant single-domain antibody fragment derived from camelids that specifically binds to the Fc region of IgG from multiple species, avoiding cross-binding to other isotypes such as IgA and IgM. These magnetic beads provide a fast, gentle method for the separation of exosomes when combined with antibodies targeting exosomal markers.

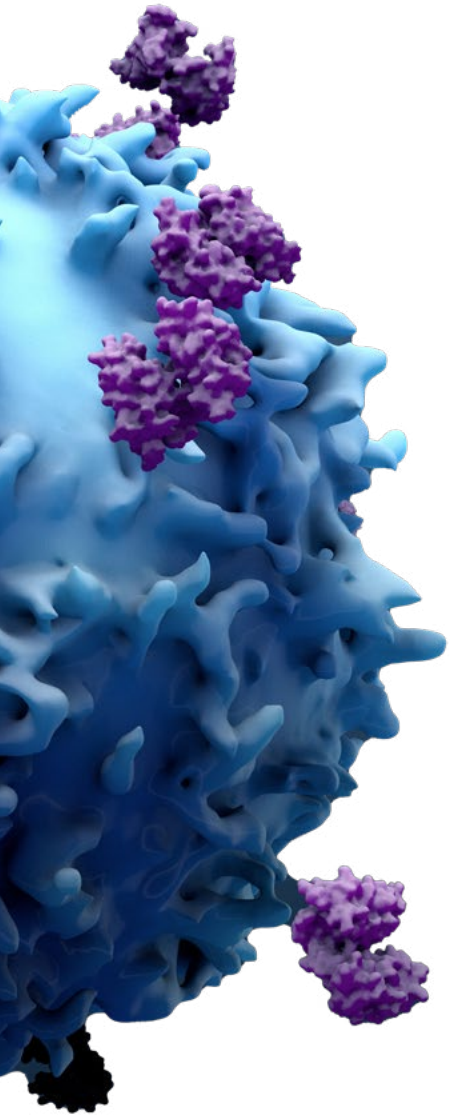
Key advantages of using DynaGreen CaptureSelect Anti-IgG-Fc magnetic beads for separating exosomes are:

- **Speed:** Rapid protocol for efficient separation of exosome subset
- **Purity:** High purity of the separated exosome subset
- **Flexibility:** Easy scale-up and automation
- **Green technology:** Microplastic-free design for sustainability, incorporating green chemistry and engineering principles

Exosome enrichment and separation using DynaGreen magnetic beads separation technology is highly scalable and allows the extraction of a highly pure set of exosomes tailored to specific research needs. We have developed a rapid bead-based system, both manual and automated (Figure 4), to enrich EVs for multiomic research.

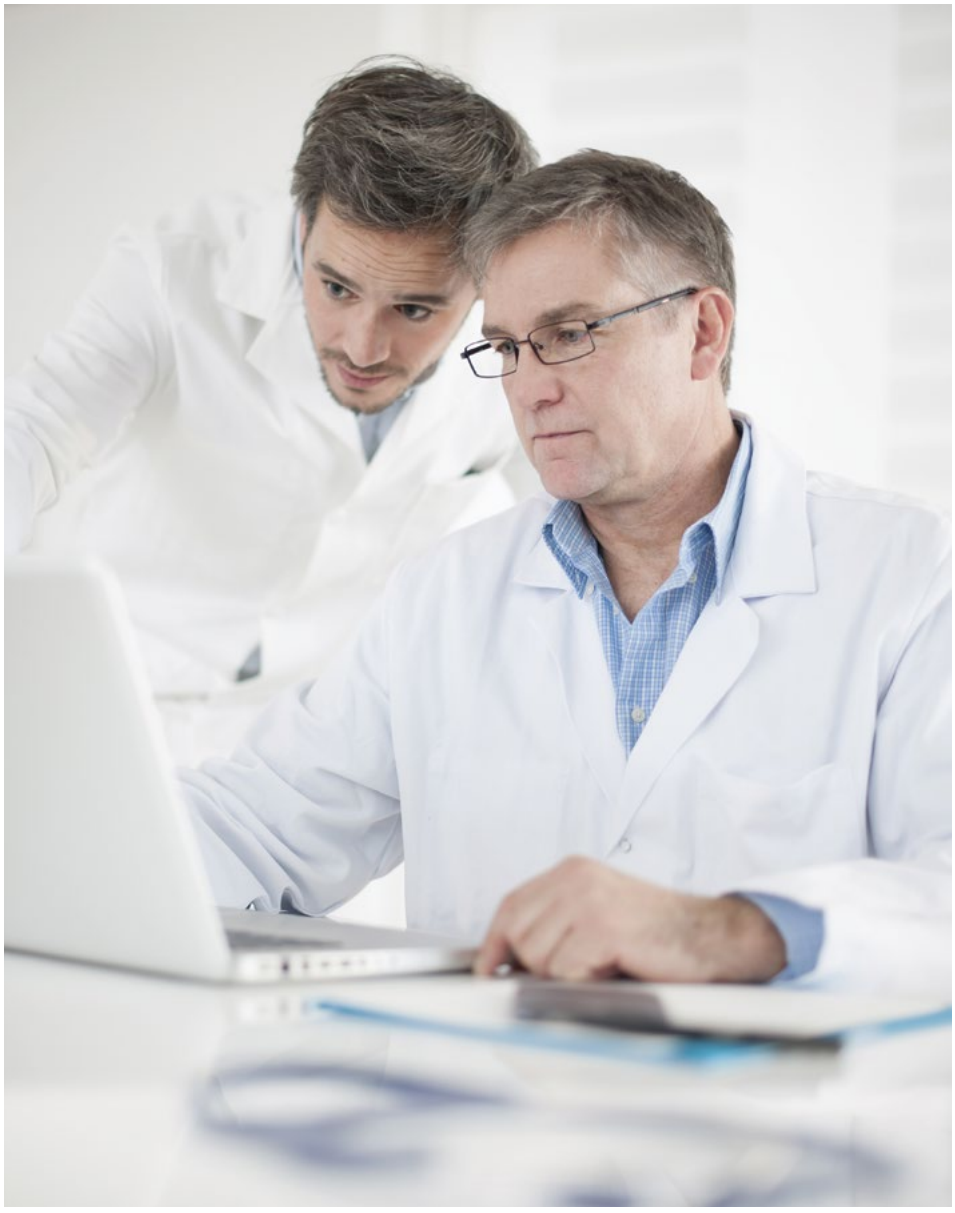
Figure 4. Exosome enrichment workflow using a manual (A) or automated (B) method.

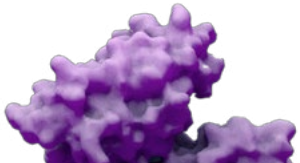
The combination of RNA and protein isolation from exosomes allows for comprehensive analysis, enabling researchers to study the full spectrum of molecular information from a single sample. Invitrogen™ Total Exosome RNA & Protein Isolation Kit efficiently isolates both RNA and protein from enriched exosomes or separated subpopulations of exosomes, making it a superb choice for multiomic studies. The process begins with the organic extraction of a portion of the sample, followed by RNA immobilization on glass fiber filters for RNA purification. The remaining sample is used for protein applications like Western blotting. With a processing time of 30 to 60 minutes, this kit provides high yields of pure RNA suitable for various downstream applications, including qRT-PCR, high-throughput sequencing, microarray analysis, and hybridization assays.



Find more information on exosome products for enrichment, separation, detection, and analysis.

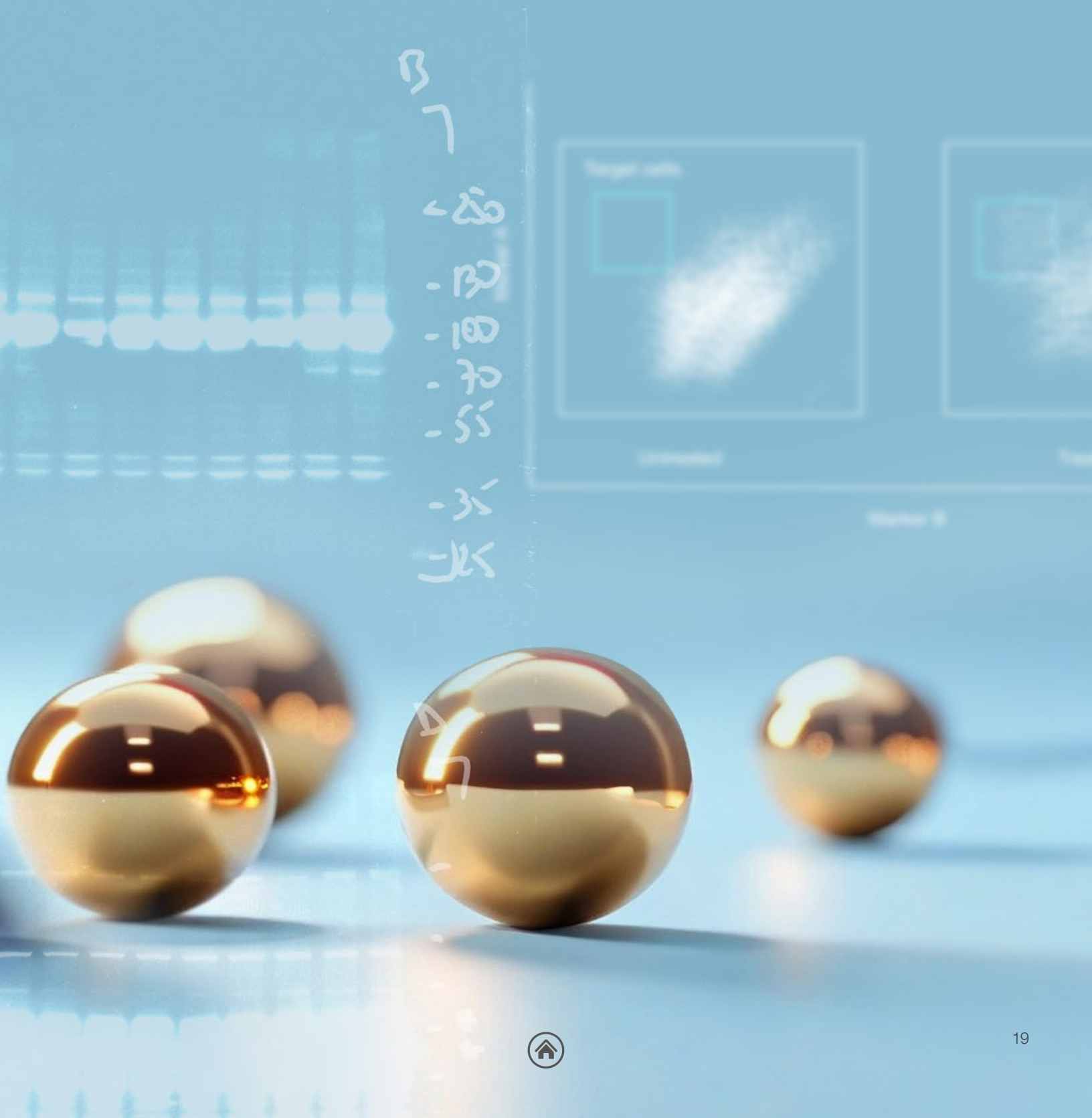
Visit thermofisher.com/exosomes





Data corner

Downstream analysis of enriched and separated exosomes



Size and phenotype

Dynabeads Intact Virus
Enrichment bead-based isolation

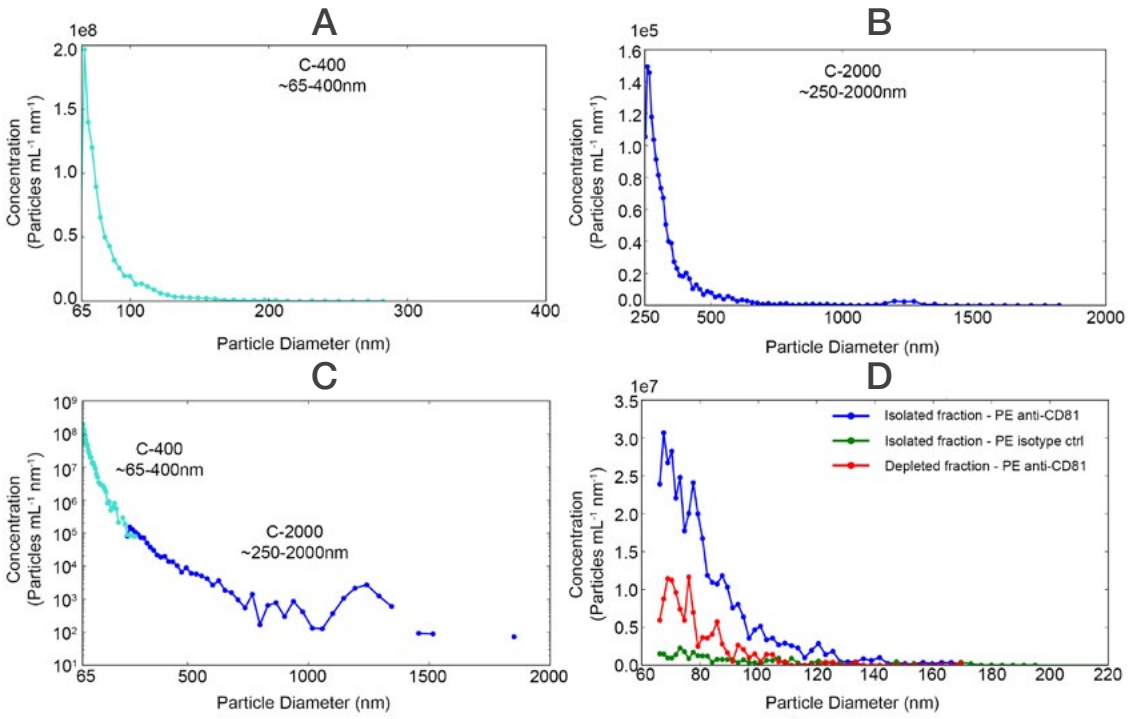


Figure 5. Multiomic analysis. Exosomes were enriched with the positively charged Dynabeads Intact Virus Enrichment beads, followed by release with 0.25 M KI in 20 mM triethanolamine. Size and concentration were measured with the Spectradyne nCS1 Particle Analyzer (A–C) according to the manufacturer's 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).

Size and phenotype, continued

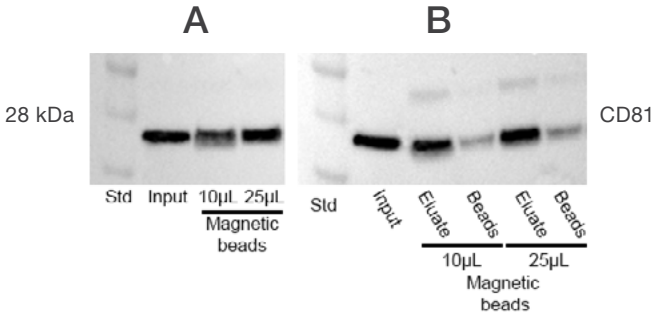


Figure 6. Enrichment of exosomes with Dynabeads Intact Virus Enrichment beads. (A) Exosomes were enriched with 10 µL or 25 µL of beads and analyzed for CD81 by western blot. (B) Exosomes were enriched with 10 µL or 25 µL of beads, followed by release. The released and unreleased exosomes were analyzed for CD81 by western blot. Std = Invitrogen™ SeeBlue™ Plus2 Pre-stained Protein Standard.

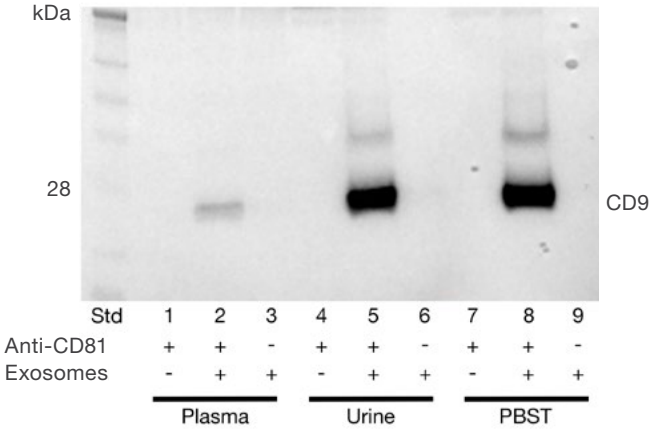


Figure 7. Enrichment of exosomes from different samples. CD81 positive exosomes were enriched from plasma, urine or PBST by DynaGreen Anti-IgG Fc magnetic beads conjugated with anti-CD81 antibodies and prepared for western blot. Identity was confirmed by detection of CD9.

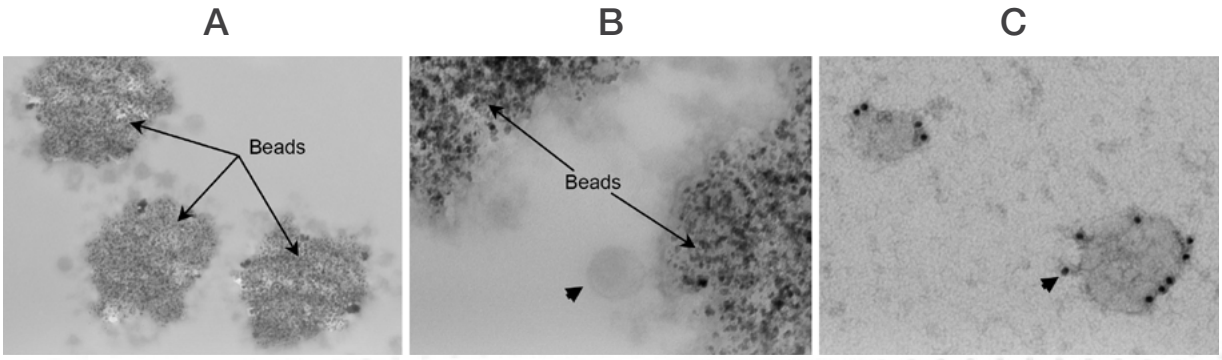


Figure 8: Ultrastructural analysis of exosomes enriched with Dynabeads Intact Virus Enrichment beads. Exosomes were enriched with strong anion exchange magnetic beads and processed for (A, B) transmission electron microscopy or (C) transmission electron microscopy in combination with immunolabeling and 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).

Flow cytometry

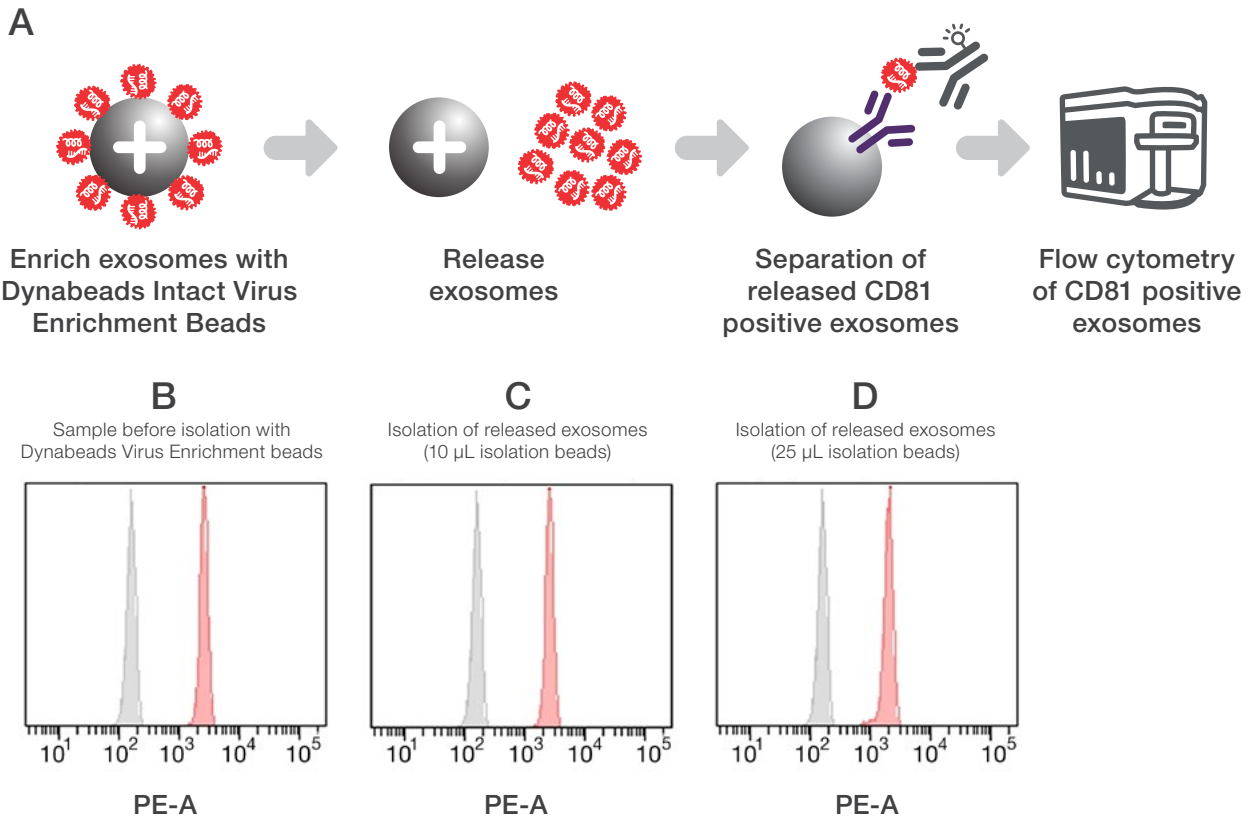


Figure 9. Flow cytometry analysis of exosomes released from Dynabeads Intact Virus Enrichment beads. (A) Exosomes were enriched from spiked samples (in PBS) with 10 µL or 25 µL of beads. The enriched exosomes were released from the beads with 0.25 M of KI and recaptured with Exosome-Human CD81 Flow Detection 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 enrichment. Exosomes released after enrichment 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.

Flow cytometry, continued

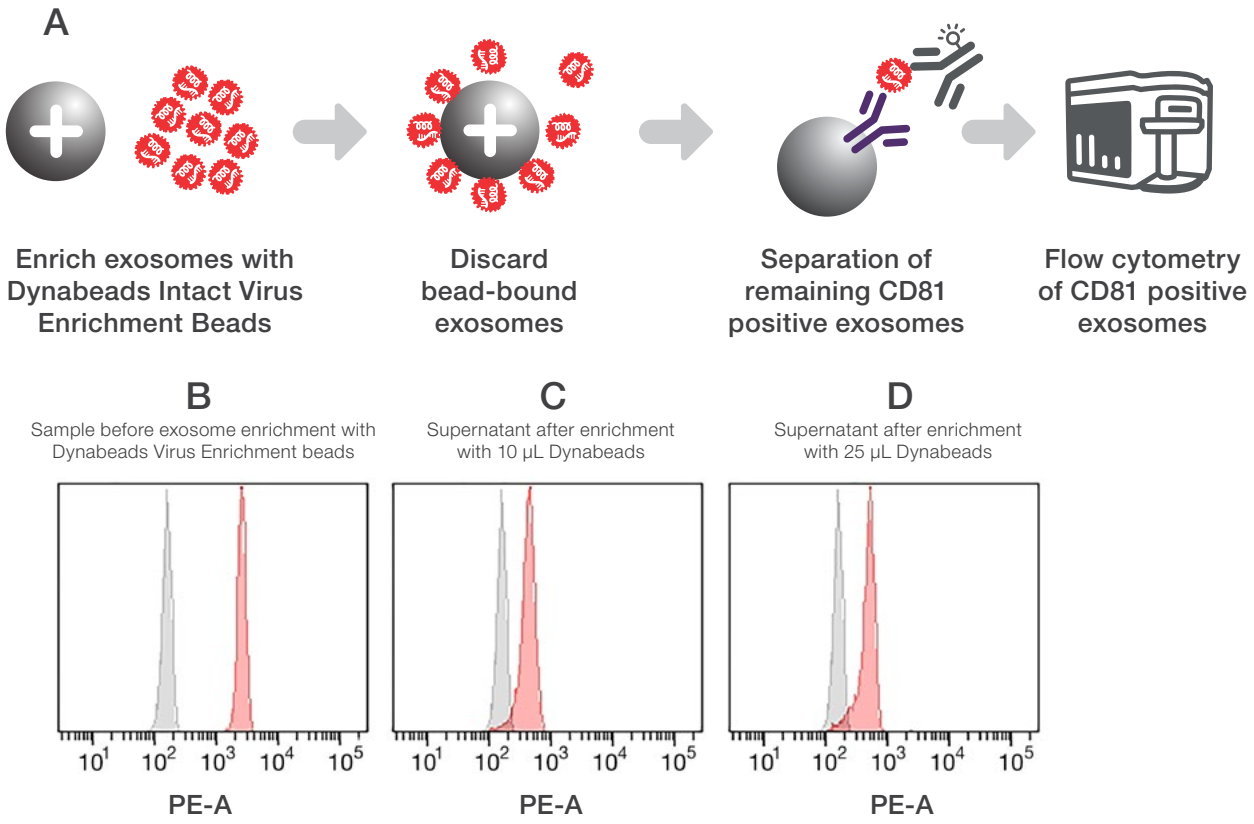


Figure 10. Flow cytometry analysis of enrichment efficiency. (A) Exosomes were enriched from spiked samples (in PBS) with 10 µL or 25 µL of Dynabeads Intact Virus Enrichment beads. The bead-bound exosomes were then discarded. The remaining exosomes in the supernatant were enriched with Exosome-Human 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 enrichment with Dynabeads Intact Virus Enrichment beads. Exosomes left in the sample after enrichment of exosomes with (C) 10 µL or (D) 25 µL of beads. The gray histograms represent the isotype control (B–D).

Flow cytometry, continued

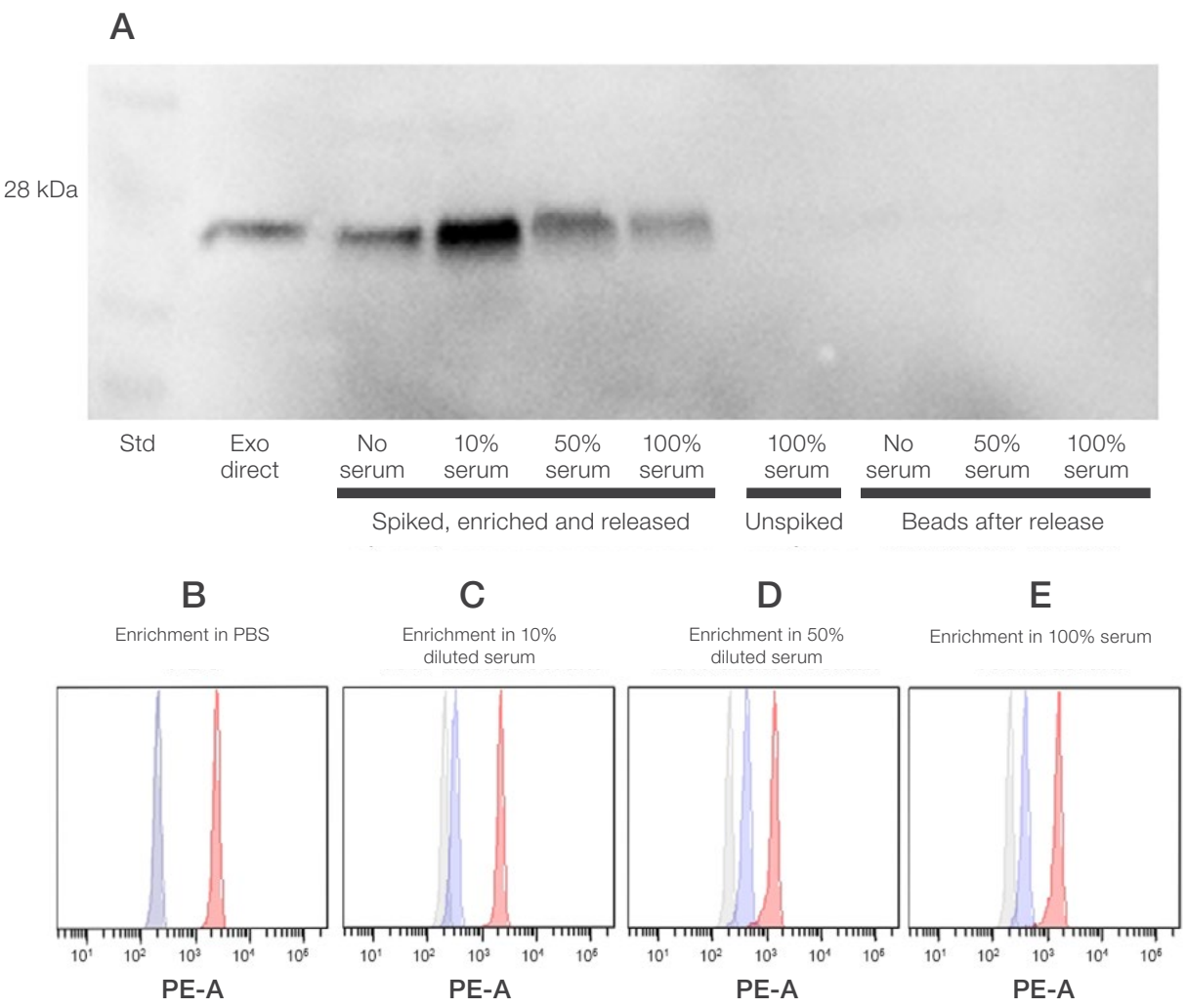


Figure 11: Enrichment of exosomes from spiked and unspiked serum with Dynabeads Intact Virus Enrichment beads. (A) Exosomes were enriched from samples of 10%, 50%, or 100% serum in buffer (PBS). The enriched exosomes were released from the beads and analyzed for CD81 by western blot. For comparison, exosomes spiked into PBS, unspiked serum, and beads after release were included. (B–E) Flow cytometry analysis of exosomes released from the beads after enrichment 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 enrichment with an irrelevant antibody, blue peaks represent unspiked samples, and red peaks represent spiked samples.

Exosome enrichment and detection products

Table 4. Exosome enrichhment and detection products

Product	Cat. No
Detection/Isolation/Immunoprecipitation	
Exosome-Human CD63 Isolation/Detection Reagent (from cell culture media)	10606D
Exosome-Streptavidin Isolation/Detection Reagent	10608D
Exosome-Human CD9 Isolation Reagent (from cell culture)	10614D
Exosome-Human CD81 Isolation Reagent (from cell culture)	10616D
Exosome-Human EpCAM Isolation Reagent (from cell culture)	10618D
Exosome-Human CD9 Flow Detection Reagent (from cell culture)	10620D
Exosome-Human CD81 Flow Detection Reagent (from cell culture)	10622D
Monoclonal antibody	
CD9 Monoclonal Antibody (Ts9)	10626D
CD63 Monoclonal Antibody (Ts63)	10628D
CD81 Monoclonal Antibody (M38)	10630D
Virus precipitation	
Intact Virus Precipitation Reagent	10720D
Isolation kits	
Total Exosome RNA & Protein Isolation Kit	4478545
Total Exosome Isolation Kit (from plasma)	4484450
Total Exosome Isolation Reagent (from urine)	4484452
Total Exosome Isolation Reagent (from other body fluids)	4484453
Total Exosome Isolation Reagent (from cell culture media)	4478359
Total Exosome Isolation Reagent (from serum)	4478360
Magnetic beads	
Dynabeads™ Intact Virus Enrichment (2 mL/10 mL)	10700D
	10701D
DynaGreen™ CaptureSelect™ Anti-IgG-Fc (Multi-Species) Magnetic Beads (0.5 mL/3 mL/10 mL)	80107G
	80108G
	80109G

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